TITLE OF THE INVENTION

SUBSTITUTED AMINO ACIDS AS ERYTHROPOIETIN MIMETICS

This invention relates to a series of small molecules which bind to the erythropoietin receptor and compete with the natural ligand for binding to said receptor. The invention includes pharmaceutical compositions containing these mimetics, their methods of production as well as intermediates used in their synthesis.

Erythropoietin (EPO) is a 34,000 dalton glycoprotein hormone which is produced in the mammalian kidney. Its primary role is stimulation of mitotic cell division and differentiation of erythrocyte precursor cells. As a result this hormone regulates the production of erythrocytes, the hemoglobin contained therein and the blood's ability to carry oxygen. The commercial product Epogen ® is used in the treatment of anemia. This drug is produced by recombinant techniques and is formulated in aqueous isotonic sodium chloride/sodium citrate. Even though it has been used successfully in the treatment of anemia, it is a costly drug that is administered intravenously. This method of administration is both costly and inconvenient for the patient; therefore it would be desirable to find a EPO mimetic which has the potential for oral activity.

A small molecule EPO mimetic has advantages over the natural protein. The immune response associated with large peptides is unlikely to occur with small molecules. In addition, the variety of pharmaceutical formulations that may be used with small molecules are technically unfeasible for proteins. Thus the use of relatively inert formulations for small molecules is possible. The most important advantage of small molecules is their potential for oral activity. Such an agent would ease administration, cost less and facilitate patient compliance.

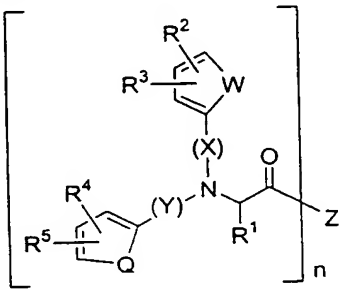
Although compounds which mimic EPO are useful in stimulating red blood cell synthesis, there are diseases where the overproduction of red blood cells is a problem.

Erythroleukemia and polysythemia vera are examples of such diseases. Since EPO is an agent responsible for the maturation of red blood cell precursors, an antagonist of EPO would have utility treating either of those diseases.

SUMMARY OF THE INVENTION

The disclosed invention consists of a series of small molecules which demonstrate competitive binding with the natural ligand for the EPO receptor. As such these compounds are potentially useful in the treatment of diseases or conditions associated with this receptor. In addition, the invention contemplates methods of producing these compounds and intermediates used in their production.

The invention includes compounds of the Formula I:



I

wherein:

R^1 is the side chain of a natural or unnatural α-amino acids, where if said side chain contains a protectable group, that group may be protected with a member of the group consisting of succinyl, glutaryl, 3,3-dimethylglutaryl, $C_{1\text{-}5}$alkyl, $C_{1\text{-}5}$alkoxycarbonyl, acetyl, N-(9-fluorenylmethoxycarbonyl), trifluoroacetyl, omega-carboxy$C_{1\text{-}5}$alkylcarbonyl, *t*-butoxycarbonyl, benzyl, benzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, phenylsulfonyl,

ureido, *t*-butyl, cinnamoyl, trityl, 4-methyltrityl, 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, tosyl, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, phenylureido, and substituted phenylureido (where the phenyl substituents are phenoxy, halo, C_{1-5}alkoxycarbonyl);

R^2 and R^3

may be taken together to form a six-membered aromatic ring which is fused to the depicted ring, or

are independently selected from the group consisting of hydrogen, C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, amino, phenyl, phenoxy, phenylC_{1-5}alkyl, phenyl C_{1-5}alkoxy,

substituted phenyl (where the substituents are selected from C_{1-5}alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenoxy (where the substituents are selected from C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenylC_{1-5}alkyl (where the substituents are selected from C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenylC_{1-5}alkoxy (where the substituents are selected from C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino), and

substituted amino (where the substituents are selected from one or more members of the group consisting of C_{1-5}alkyl, halosubstitutedC_{1-5}alkyl, C_{1-5}alknyl, C_{1-5}alkenyl, phenyl, phenylC_{1-5}alkyl, C_{1-5}alkylcarbonyl, halo substituted

C_{1-5}alkylcarbonyl, carboxyC_{1-5}alkyl, C_{1-5}alkoxyC_{1-5}alkyl, cinnamoyl, naphthylcarbonyl, furylcarbonyl, pyridylcarbonyl, C_{1-5}alkylsulfonyl, phenylcarbonyl, phenylC_{1-5}alkylcarbonyl, phenylsulfonyl, phenylC_{1-5}alkylsulfonyl substituted phenylcarbonyl, substituted phenylC_{1-5}alkylcarbonyl, substituted phenylsulfonyl, substituted phenylC_{1-5}alkylsulfonyl, substituted phenyl, and substituted phenylC_{1-5}alkyl [where the aromatic phenyl, phenylC_{1-5}alkyl, phenylcarbonyl, phenylC_{1-5}alkylcarbonyl, phenylsulfonyl, and phenylC_{1-5}alkylsulfonyl substitutents are independently selected from one to five members of the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, hydroxy, halogen, trifluoromethyl, nitro, cyano, and amino]);

R^4 and R^5

may be taken together to form a six-membered aromatic ring which is fused to the depicted ring, or

are independently selected from the group consisting of hydrogen, C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, amino, phenyl, phenoxy, phenylC_{1-5}alkyl, phenyl C_{1-5}alkoxy,

substituted phenyl (where the substituents are selected from C_{1-5}alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenoxy (where the substituents are selected fromC_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenylC_{1-5}alkyl (where the substituents are selected from C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino),

substituted phenylC_{1-5}alkoxy (where the substituents are selected from C_{1-5} alkyl, C_{1-5} alkoxy, hydroxy, halo, trifluoromethyl, nitro, cyano, and amino), and

substituted amino (where the substituents are selected from one or more members of the group consisting of C_{1-5}alkyl, halosubstitutedC_{1-5}alkyl, C_{1-5}alknyl, C_{1-5}alkenyl, phenyl, phenylC_{1-5}alkyl, C_{1-5}alkylcarbonyl, halo substituted C_{1-5}alkylcarbonyl, carboxyC_{1-5}alkyl, C_{1-5}alkoxyC_{1-5}alkyl, cinnamoyl, naphthylcarbonyl, furylcarbonyl, pyridylcarbonyl, C_{1-5}alkylsulfonyl, phenylcarbonyl, phenylC_{1-5}alkylcarbonyl, phenylsulfonyl, phenylC_{1-5}alkylsulfonyl substituted phenylcarbonyl, substituted phenylC_{1-5}alkylcarbonyl, substituted phenylsulfonyl, substituted phenylC_{1-5}alkylsulfonyl, substituted phenyl, and substituted phenylC_{1-5}alkyl [where the aromatic phenyl, phenylC_{1-5}alkyl, phenylcarbonyl, phenylC_{1-5}alkylcarbonyl, phenylsulfonyl, and phenylC_{1-5}alkylsulfonyl substitutents are independently selected from one to five members of the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, hydroxy, halogen, trifluoromethyl, nitro, cyano, and amino]);

W is selected from the group consisting of -CH=CH-, -S-, and -CH=N-;

Q is selected from the group consisting of -CH=CH-, -S-, and -CH=N-;

X is selected from the group consisting of carbonyl, C_{1-5}alkyl, C_{1-5}alkenyl, C_{1-5}alkenylcarbonyl, and $(CH_2)_m$-C(O)- where m is 2-5;

Y is selected from the group consisting of carbonyl, C_{1-5}alkyl, C_{1-5}alkenyl, C_{1-5}alkenylcarbonyl, and $(CH_2)_m$-C(O)- where m is 2-5;

n is 1, 2, or 3;

Z is selected from the group consisting of hydroxy, C_{1-5} alkoxy, phenoxy, phenylC_{1-5}alkoxy, amino, C_{1-5}alkylamino, diC_{1-5}alkylamino, phenylamino, phenylC_{1-5}alkylamino,

piperidin-1-yl

substituted piperidin-1-yl (where the substituents are selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, halo, aminocarbonyl, C_{1-5}alkoxycarbonyl, and oxo;

substituted phenylC_{1-5}alkylamino (where the aromatic substitutents are selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, phenylC_{1-5}alkenyloxy, hydroxy, halogen, trifluoromethyl, nitro, cyano, and amino),

substituted phenoxy (where the aromatic substitutents are selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, hydroxy, halogen, trifluoromethyl, nitro, cyano, and amino),

substituted phenylC_{1-5}alkoxy (where the aromatic substitutents are selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, hydroxy, halogen, trifluoromethyl, nitro, cyano, and amino),

$-OCH_2CH_2(OCH_2CH_2)_sOCH_2CH_2O-$,

$-NHCH_2CH_2(OCH_2CH_2)_sOCH_2CH_2NH-$,

$-NH(CH_2)_pO(CH_2)_qO(CH_2)_pNH-$, $-NH(CH_2)_qNCH_3(CH_2)_sNH-$, $-NH(CH_2)_sNH-$, and $(NH(CH_2)_s)_3N$,

where s, p, and q are independently selected from 1-7

with the proviso that if n is 2, Z is not hydroxy, C_{1-5} alkoxy, amino, C_{1-5}alkylamino, diC_{1-5}alkylamino, phenylamino, phenylC_{1-5}alkylamino, or piperidin-1-yl,

with the further proviso that if n is 3, Z is $(NH(CH_2)_s)_3N$.

and the salts thereof.

DETAILED DESCRIPTION OF THE INVENTION

The terms used in describing the invention are commonly used and known to those skilled in the art. "Independently" means that when there are more than one substituent, the substitutents may be different. The term "alkyl" refers to straight, cyclic and branched-chain alkyl groups and "alkoxy" refers O-alkyl where alkyl is as defined supra. "Cbz" refers to benzyloxycarbonyl. "Boc" refers to t-butoxycarbonyl and "Ts" refers to toluenesulfonyl. "DCC" refers to 1,3-dicyclohexylcarbodiimide, "DMAP" refers to 4-N',N-dimethylaminopyridine and "HOBT" refers to 1-hydroxybenzotriazole hydrate. "Fmoc" refers to N-(9-fluorenylmethoxycarbonyl), "DABCO" refers to 1,4-Diazabicyclo[2.2.2]octane, "EDCI" refers to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, "Dde" refers to 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, and "TMOF" refers to trimethyl orthoformate. The side chains of α-amino acids refer to the substituents of the stereogenic carbon of an α-amino acid. For example if the amino acid is lysine, the side chain is 1-aminobutan-4-yl. The term natural amino acid refers to the 20 α-amino acids of the L configuration which are found in natural proteins. Unnatural α-amino acids include synthetic amino acids such as , α-aminoadipic acid, 4-aminobutanoic acid, 6-aminohexanoic acid, α-aminosuberic acid, 5-aminopentanoic

acid, p-aminophenylalanine, α-aminopimelic acid γ-carboxyglutamic acid, p-carboxyphenylalanine, carnitine, citrulline, α,β-diaminopropionic acid, α,γ-diaminobutyric acid, homocitrulline, homoserine, and statine as well as D-configuration amino acids. The term "protectable group" refers to a hydroxy, amino, carboxy, carboxamide, guanidine, amidine or a thiol groups on an amino acid side. Compounds of the invention may be prepared by following general procedures known to those skilled in the art, and those set forth herein.

The compounds of the invention may be prepared by liquid phase organic synthesis techniques or by using amino acids which are bound to a number of known resins. The underlying chemistry, namely, acylation and alkylation reactions, peptide protection and deprotection reactions as well as peptide coupling reactions use similar conditions and reagents. The main distinction between the two methods is in the starting materials. While the starting materials for the liquid phase syntheses are the N-protected amino acids or the lower alkyl ester derivatives of either the N-protected or N-unprotected amino acids, the starting material for the resin syntheses are N-protected amino acids which are bound to resins by their carboxy termini.

General Procedure For The Solid-Phase Synthesis Of Symmetrical Nα,Nα-Disubstituted Amino Acids

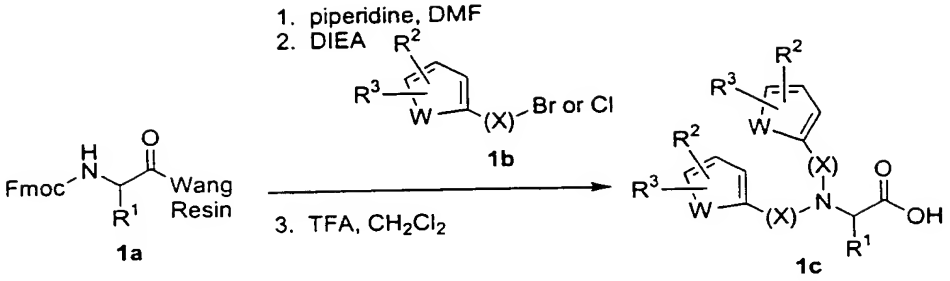
Scheme 1

An equivalent of an N-Fmoc-protected amino acid which is bound to a resin **1a** is suspended in a suitable solvent such as DMF. This solvent is removed and the nitrogen protecting group (Fmoc) is removed by stirring the resin bound amino acid with an organic base, such as piperidine, and an addition portion of the solvent. A solution of about two to three equivalents of an appropriately substituted halide, **1b**, and a suitable base such DIEA is added to the resin bound amino acid and this mixture is shaken for 18-36 h. The resulting mixture is washed with several portions of a suitable solvent and is suspended and shaken in an acidic solution, such

as 50% TFA/CH_2Cl_2, over several hours to cleave the acid from the resin and give the *N*-disubstituted amino acid **1c**.

By varying the resin bound amino acid **1a**, one may obtain many of the compounds of the invention. The following resin bound amino acids may be used in Scheme I: alanine, N-g-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)arginine, β-(4-methyltrityl)asparagine, aspartic acid (β-t-butyl ester), S-(trityl)cysteine, γ-(4-methyltrityl)glutamine, glutamic acid (β-t-butyl ester), glycine, N-imidazolyl-(trityl)histidine, isoleucine, leucine, N-ε-(2-chlorobenzyloxycarbonyl)lysine, N-ε-(t-butoxycarbonyl)lysine, methionine, phenylalanine, proline, O-(t-butyl)serine, O-(t-butyl)threonine, N-indolyl-(t-butoxycarbonyl)tryptophan, O-(t-butyl)tyrosine, valine, β-alanine, α-aminoadipic acid, 4-aminobutanoic acid,, 6-aminohexanoic acid, α-aminosuberic acid, 5-aminopentanoic acid, p-aminophenylalanine, α-aminopimelic acid γ-carboxyglutamic acid, p-carboxyphenylalanine, carnitine, citrulline, α,β-diaminopropionic acid, α,γ-diaminobutyric acid, homocitrulline, homoserine, and statine. In addition, the choice of "W" and "X" can be varied by using known halide derivatives of **1b**. For example using benzylchloride, 2-chloromethylthiophene, or 2-chloromethylpyridine gives compounds of the invention where "W" is -CH=CH-, -S-, or -CH=N-, respectively. For variations in "X", the use of 2-chloroethylphenyl, 3-chloro-1-propenylbenzene, or benzeneacetyl chloride as **1b**, give compounds where Y is $(CH_2)_2$, -CH=CH-CH_2-, or -CH_2C(O)- respectively. Still further, Scheme 1 may be used to produce combinatorial mixtures of products. Using mixtures of resin bound amino acids, **1a**, with only one **1b** produces said combinatorial mixtures. Alternatively, using one amino acid **1a** with a mixture of **1b** as well as mixture of **1a** with mixtures of **1b** gives a large range of combinatorial mixtures.

Scheme 1



General Procedure For The Solid-Phase Synthesis Of Unsymmetrical Nα,Nα-Disubstituted Amino Acids

Scheme 2, Step A

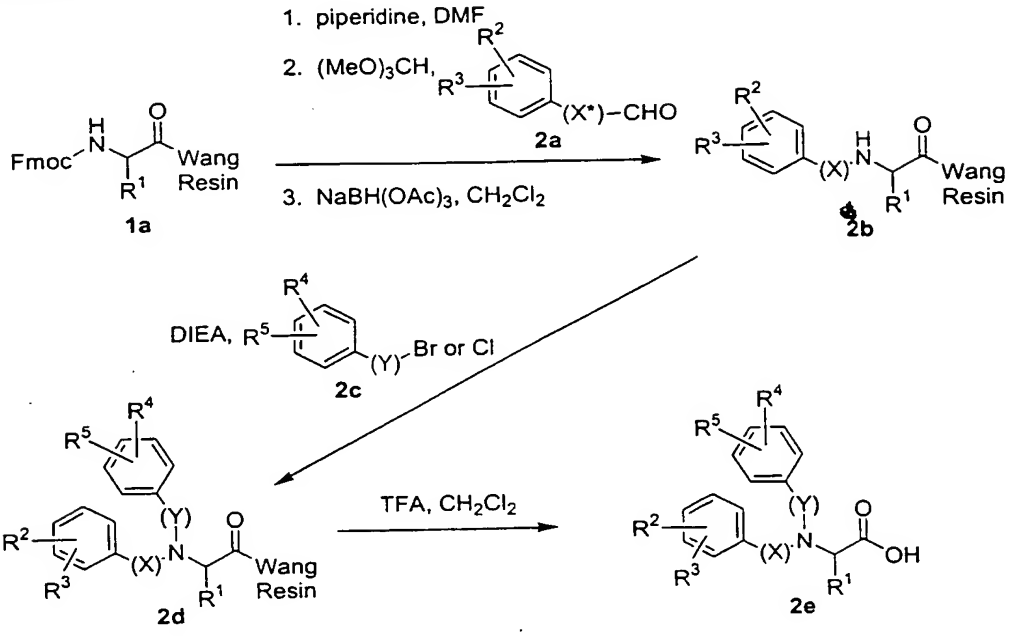
An equivalent of an N-Fmoc-protected amino acid which is bound to a resin **1a** is suspended in a suitable solvent such as DMF. This solvent is removed and the nitrogen protecting group (Fmoc) is removed by stirring the resin bound amino acid with an organic base, such as piperidine, and an addition portion of the solvent. Trimethyl orthoformate and an appropriately substituted aldehyde **2a** (5 equivalents) is added and the mixture is shaken under N_2 overnight. This mixture is treated with a suspension of $NaBH(OAc)_3$ (5 equivalents) in CH_2Cl_2 and shaken under N_2 overnight. After filtration and washing with a suitable solvent, the resulting product, resin bound Nα-monosubstituted amino acid **2b**, is rinsed with a suitable solvent and its identity is confirmed by MS and or HPLC analysis after treatmet of a portion of the resin with 50% TFA/CH_2Cl_2.

Scheme 2, Step B

The resin **2b** is suspended in an appropriate solvent such as DMF and is filtered. The appropriately substituted alkyl or arylkyl halide, **2c**, and an appropriate base such as DIEA are added with some additional solvent and the mixture is shaken under N_2 for 18–36 h. The resin bound Nα,Nα-disubstituted amino acid, **2d**, is

isolated from the suspension and the resin is cleaved with an acidic solution to give the free acid **2e**.

Scheme 2



Scheme 3, Step C

A resin bound amine, **2d**, where R^4 is nitro, is suspended in a suitable solvent, such as DMF, and is filtered. This mixture is treated with $SnCl_2$ dihydrate in DMF and shaken under N_2 overnight. The solvent is removed and the resin is washed successive portions of a suitable solvent to give the resin bound compound **3a** where R^4 is amino. The resin is suspended in a suitable solvent and is combined with an organic base, such as pyridine an appropriately substituted carboxylic acid anhydride, acid chloride, or sulfonyl chloride. The mixture is shaken under N_2

overnight and is filtered to give the resin bound amino acid **3b**. This material is treated with an acid and a suitable solvent to give the free amino acid **3b**.

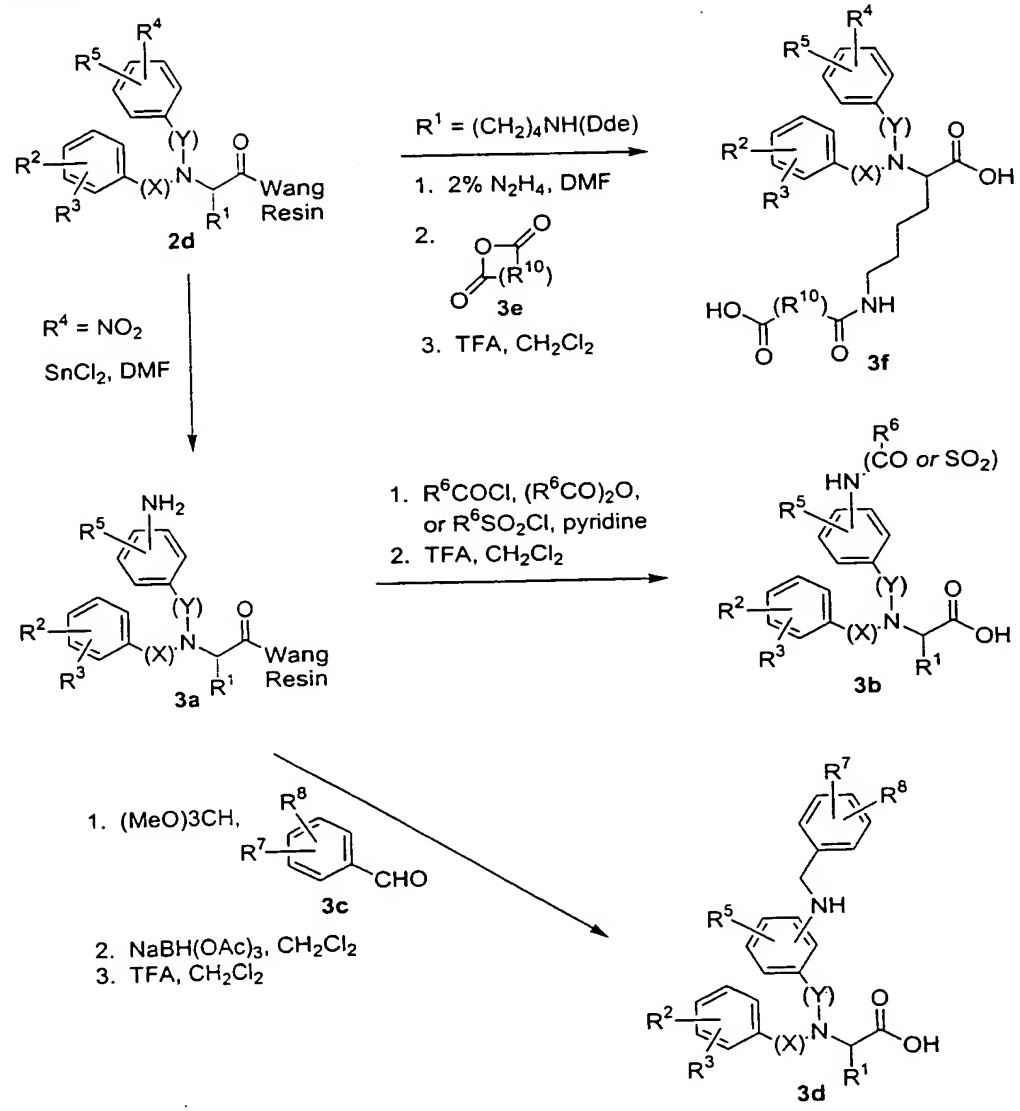
Scheme 3, Step D

The resin bound amine **3a** is treated with TMOF and an appropriately substituted aldehyde **3c** is added and the mixture is shaken under N_2 overnight. The resulting mixture is drained and treated with a suspension of $NaBH(OAc)_3$ in an appropriate solvent and this mixture is shaken under N_2 overnight. The resin bound 3-aralkylaminophenyl amino acid is identified my spectral techniques after clevage to give the free acid **3d** as previously described.

Scheme 3, Step E

Resin bound, **2d**, where R^1 is $(CH_2)_4NH(Dde)$ is mixed with a suitable solvent, such as DMF, and shaken with successive portions of 2% solution of hydrazine hydrate in DMF over about 30 min. The resin is filtered and treated with a suitable solvent and a cyclic anhydride derivative **3e**, and a base such as DMAP and pyridine. This mixture is shaken under N_2 overnight and filtered to give the resin bound amine, **3f**. This material is identified by spectral techniques after clevage to give the free acid **3f** as previously described.

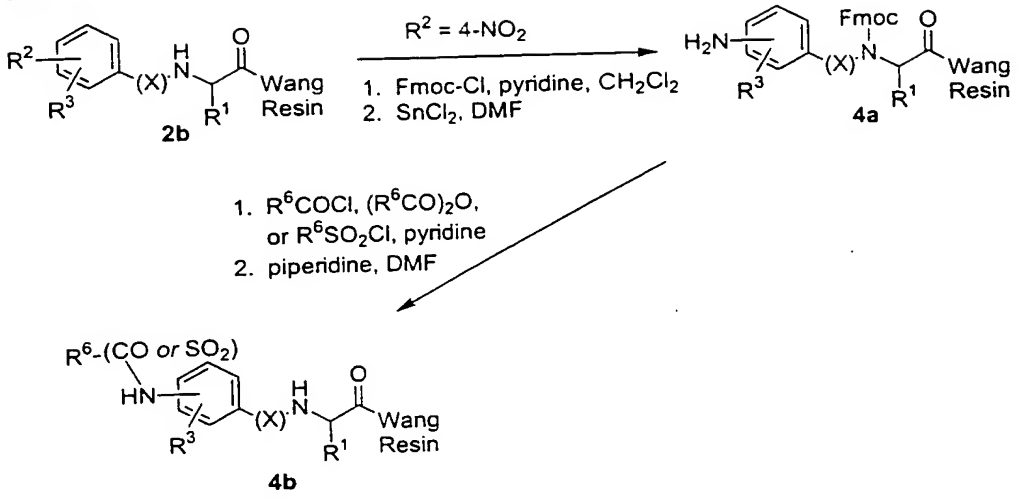
Scheme 3


R1 = (CH2)4NH(Dde)
1. 2% N2H4, DMF
2.
3e
3. TFA, CH2Cl2
2d
Wang Resin
R4 = NO2
SnCl2, DMF
3a
1. R6COCl, (R6CO)2O, or R6SO2Cl, pyridine
2. TFA, CH2Cl2
3b
1. (MeO)3CH,
3c
2. NaBH(OAc)3, CH2Cl2
3. TFA, CH2Cl2
3d
3f

Scheme 4, Step F

Resin bound **2b**, where R^2 is nitro is suspended in CH_2Cl_2 and is treated with an organic base, such as pyridine, and 9-fluorenylmethoxy chloride. This mixture is shaken under N_2 overnight, filtered and resuspended in a suitable solvent. This mixture is treated with $SnCl_2$ dihydrate in DMF and shaken under N_2 overnight. The solvent is removed and the resin is washed successive portions of a suitable solvent and filtered to give the resin bound compound **4a** where R^2 is amino. The resin **4a** is then suspended in a suitable solvent, such as CH_2Cl_2, and is combined with 0.4 mmol of pyridine and 0.25–0.4 mmol of the appropriately substituted carboxylic acid anhydride, acid chloride, or sulfonyl chloride. The mixture is shaken under N_2 overnight, filtered, and washed successively with three portions each of CH_2Cl_2 and MeOH. This resin is suspended in DMF, filtered, and shaken under N_2 with 5 mL of a 40% solution of piperidine in DMF. After 1 h, the solvent is drained and the resin was washed successively with three portions each of suitable solvents to give the resin bound **4b**. The identity of the compound was confirmed by spectral analysis after cleveage as previously described.

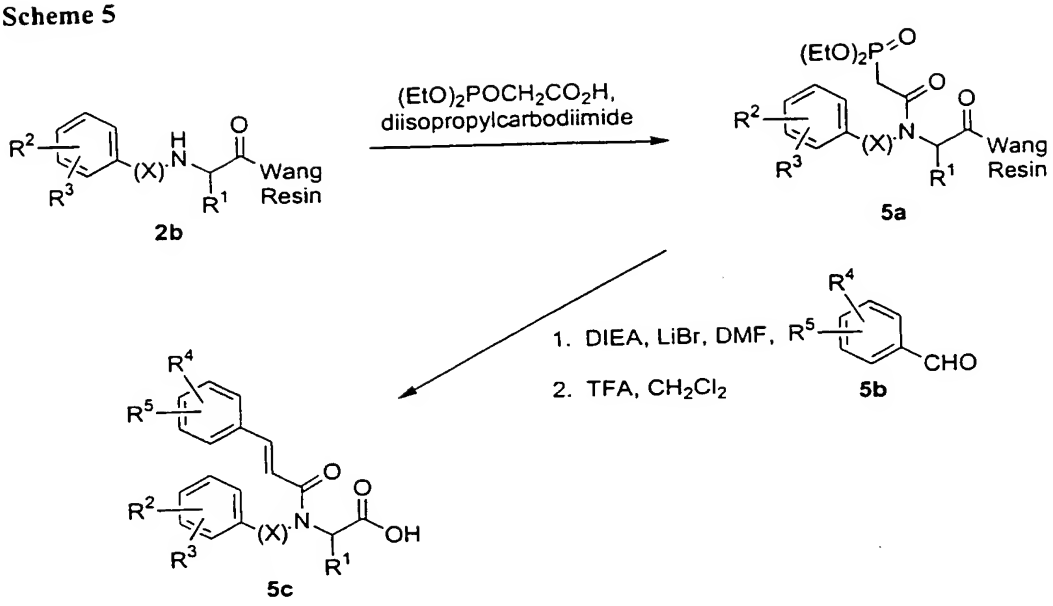
Scheme 4



Scheme 5

The resin **2b** (0.2 mmol) is suspended in CH_2Cl_2, filtered, and is resuspended in CH_2Cl_2. This suspension is treated with diethyl phosphonoacetic acid and diisopropylcarbodiimide or other suitable carbodiimide reagent, and the mixture is shaken under N_2 overnight. The solvent is drained and the resulting resin **5a** was washed successively with three portions each of CH_2Cl_2 and MeOH. The resin is suspended in DMF and filtered. A solution of the appropriately substituted aldehyde **5b** (0.6–1.0 mmol) in 3–5 mL of DMF, lithium bromide (0.6–1.0 mmol), and a suitable base such as DIEA or Et_3N (0.6–1.0 mmol) is added and the mixture is shaken under N_2 overnight. The solvent is removed and the resin is washed successively with three portions each of DMF, CH_2Cl_2, and MeOH. The identity of the resin bound substituted amino acid **5c** was confirmed spectral techniques. The resin bound material may be treated with 50% TFA/CH_2Cl_2 over 1–1.5 h, to give the acid **5c**.

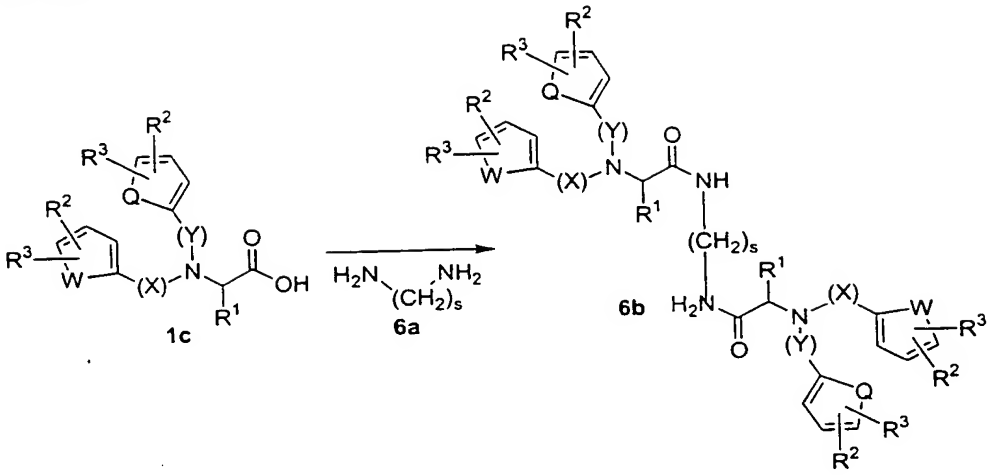
Scheme 5



Scheme 6

To prepare compounds where n is 2 and Z is $NH(CH_2)_sNH$, products of Schemes 1-5 may be used in Scheme 6. Treatment of two equivalents of the substituted amino acid **1c** with an equivalent of the diamine **6a,** in the presence of HOBT and a peptide coupling agent such as EDCI and a base such as DIEA at room temperature over 16 h gives the dimer **6b**.

Scheme 6

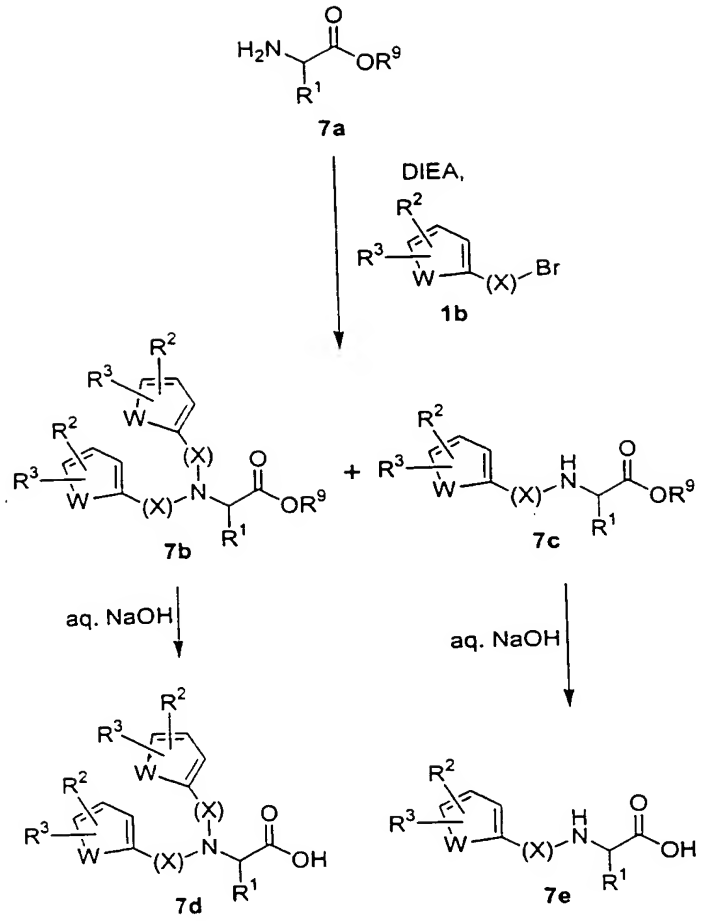


General Procedure For The Solution-Phase Synthesis Of Symmetrical Nα,Nα-Disubstituted Amino Acids

Scheme 7, Step A

A solution of of amino acid ester **7a**, an appropriately substituted halide derivitive **1b**, and an appropriate base such as DIEA, Na_2CO_3, or Cs_2CO_3 in a suitable solvent, such as DMF, is heated at 50–100 °C under N_2 overnight, or until the starting material is exhausted, to give a mixture of the di and mono-substituted amines, **7b** and **7c** respectively. If the side chains of R^1contain acid cleavable protecting groups, those groups may be cleaved by treatment with 30-80% TFA/CH_2Cl_2. Esters **7b** and **7c** may be independently converted to the corresponding acids **7d** and **7e** by hydrolysis with an appropriate base such as aqueous NaOH.

Scheme 7



General Procedure For The Solution-Phase Synthesis Of Unsymmetrical Nα,Nα-Disubstituted Amino Acids

Scheme 8, Step A

A solution of 1 mmol of amino acid ester **8a** (or the corresponding HCl salt and 1.1 mmol of DIEA) and 1–1.5 mmol of the appropriately substituted aldehyde

2a in 3–5 mL of trimethyl orthoformate was stirred at room temperature under N_2 overnight. The solution was either concentrated and used directly for the next reaction, or was partitioned between EtOAc and water, washed with brine, dried over Na_2SO_4, and concentrated to give crude product, which was purified by MPLC to give mono-substituted product **8b**.

Scheme 8, Step B

Amino ester **8b** was dissolved in DMF, combined with 1.1–1.5 mmol of the appropriately substituted chloride or bromide **2c**, and heated at 50–100 °C overnight. The reaction mixture was cooled and partitioned between water and EtOAc. The organic layer was washed three times with water and once with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by MPLC to give pure **8c**. For examples of **8c** wherein the side chain R^1 contained an acid-cleavable protecting group such as t-butylcarbamate, t-butyl ester, or t-butyl ether, **8c** was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give the deprotected form of **8c**. For examples of **8c** where R^9 was equal to t-butyl, **8c** was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h and treated as described above to give acid **8d**. For examples of **8c** where R^9 was equal to methyl, ethyl, or other primary or secondary alkyl esters, **8c** was stirred with with 1–2 mmol of aqueous LiOH, NaOH, or KOH in MeOH, EtOH, or THF at 20–80 °C until TLC indicated the absence of **8c**. The solution was acidified to pH 4–5 with aqueous citric acid or HCl and was extracted with CH_2Cl_2 or EtOAc. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated to give **8d**.

Scheme 8, Step C

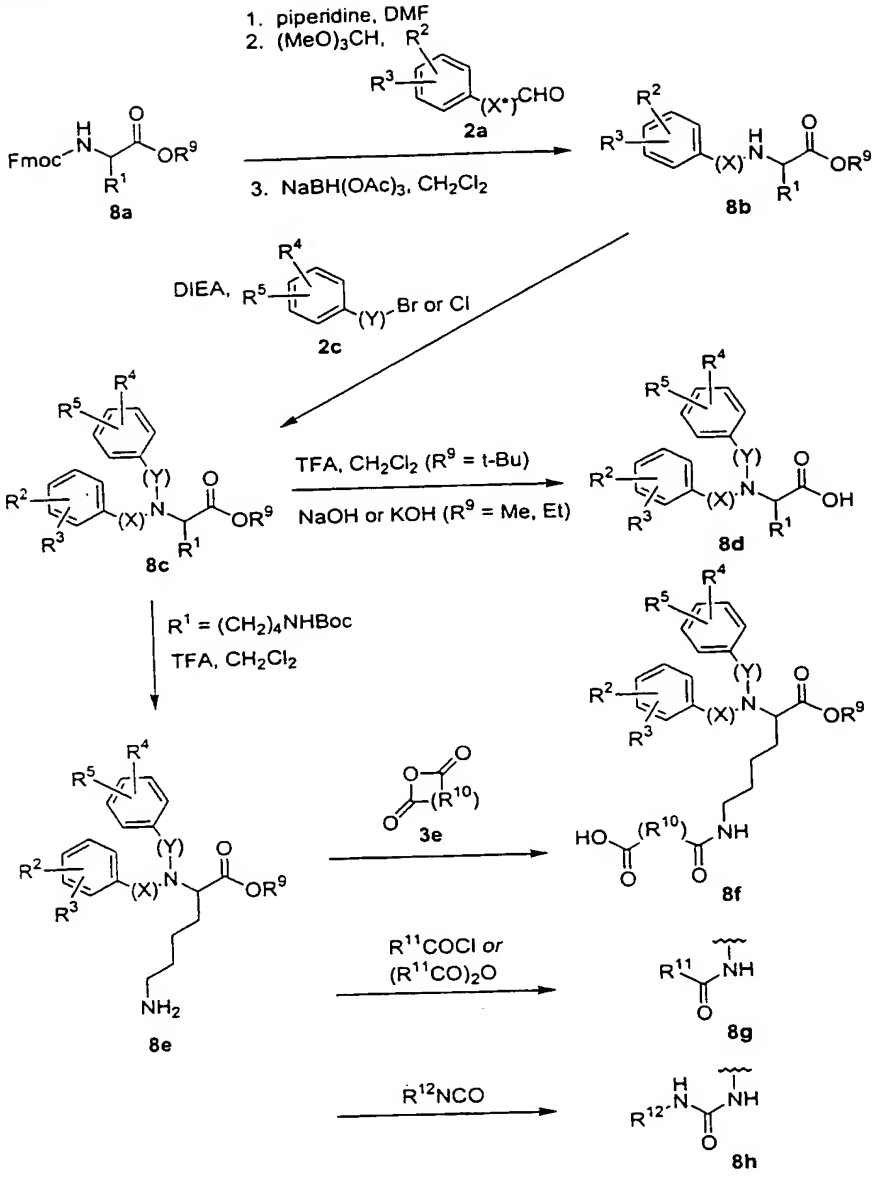
For examples of amino acid ester **8c** where $R^1 = (CH_2)_4NHBoc$, **8c** (1 mmol) was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h. The reaction mixture was concentrated to provide **8e** as the TFA salt. Optionally, the TFA salt was dissolved

in CH_2Cl_2 or EtOAc and washed with aqueous NaOH or Na_2CO_3, dried over Na_2SO_4, and concentrated to give **8e** as the free base.

Scheme 8, Step D

A solution of 1 mmol of **8e**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted cyclic anhydride **3e** was stirred in CH_2Cl_2 or DMF under N_2 overnight. The resulting mixture was diluted with CH_2Cl_2 or EtOAc and washed with aqueous HCl, water, and brine, was dried over Na_2SO_4, and concentrated to provide **8f**. Alternatively, 1 mmol of **8e**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted carboxylic acid anhydride $(R^{11}CO)_2O$ or acid chloride $R^{11}COCl$ was stirred in CH_2Cl_2 or DMF under N_2 overnight and worked up as above to provide **8g**. Alternatively, 1 mmol of **8e**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted isocyanate $R^{12}NCO$ was stirred in CH_2Cl_2 or DMF under N_2 overnight and worked up as above to provide **8h**.

Scheme 8.


1. piperidine, DMF
2. (MeO)3CH,
2a
3. NaBH(OAc)3, CH2Cl2
8a
8b
DIEA,
2c
Br or Cl
TFA, CH2Cl2 (R9 = t-Bu)
NaOH or KOH (R9 = Me, Et)
8c
8d
R1 = (CH2)4NHBoc
TFA, CH2Cl2
3e
8e
8f
R11COCl or
(R11CO)2O
8g
R12NCO
8h

Scheme 9, Step A

For examples of **8c** where $R^5 = NO_2$, a solution of 1 mmol of **8c** (where R^2, R^3, R^4, or) and 10–12 mmol of $SnCl_2$ dihydrate was stirred in MeOH, EtOH, or DMF at 20–80 °C for 0.5–24 h under N_2. The solution was taken to room temperature and poured into aqueous Na_2CO_3 with rapid stirring. The resulting mixture was extracted with EtOAc or CH_2Cl_2 and the organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to give the aminophenyl product **9a**, which was purified by MPLC or used without further purification.

Scheme 9, Step B

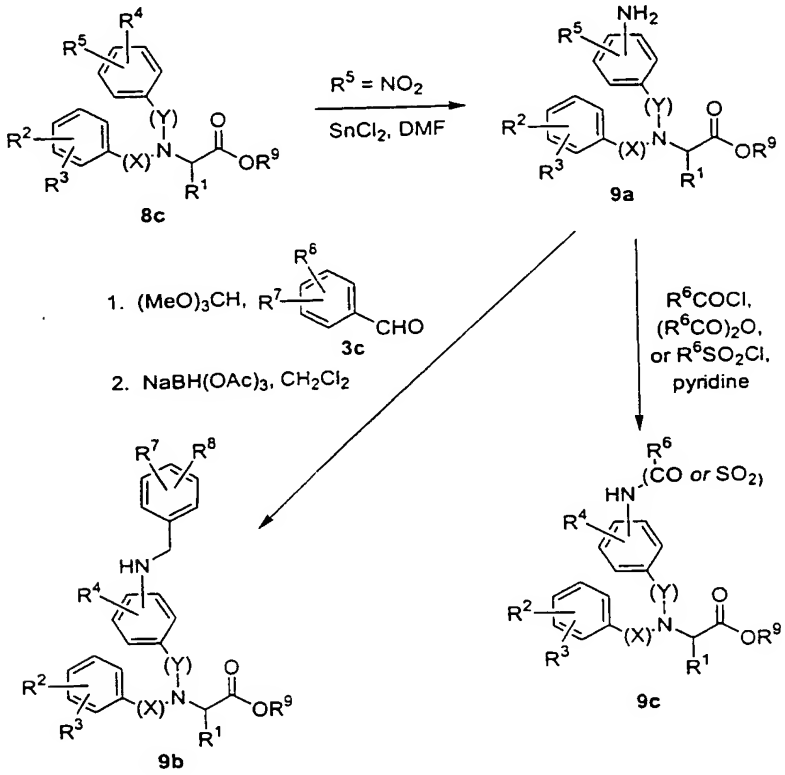
A solution of 1 mmol of aminophenyl compound **9a** and 1–1.5 mmol of the appropriately substituted aldehyde **2a** in 3–5 mL of trimethyl orthoformate was stirred at room temperature under N_2 overnight. The solution was either concentrated and used directly for the next reaction, or was partitioned between EtOAc and water, washed with brine, dried over Na_2SO_4, and concentrated to give crude product, which was purified by MPLC to give **9b**. For examples of **9b** wherein the side chain R^1 or R^9 contained an acid-cleavable protecting group such as t-butylcarbamate, t-butyl ester, or t-butyl ether, **9b** was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give the deprotected form of **9b**.

Scheme 9, Step C

A solution of 1 mmol of 3-aminophenyl compound **9a**, 1.1–2 mmol of pyridine, and 1–1.5 mmol of the appropriately substituted acid chloride, acid anhydride, or sulfonyl chloride in 3–5 mL of CH_2Cl_2 or $ClCH_2CH_2Cl$ was stirred at room temperature under N_2 overnight. The solution was partitioned between EtOAc and water, washed with water, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated to give crude product which was optionally purified by MPLC to give amide or sulfonamide **9c**. For examples of **9c** wherein the side chain R^1 or R^9 contained an acid-cleavable protecting group such as t-butylcarbamate, t-

butyl ester, or t-butyl ether, **9c** was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give the deprotected form of **9c**.

Scheme 9.



General Procedure For The Solution-Phase Synthesis Of Symmetrical Nα,Nα-Disubstituted Amino Amides And Their Dimers and Trimers

Scheme 10, Step A

A solution of 1 mmol of N-Cbz-protected amino acid **10a** and the appropriate amine (ZH, 1 mmol), diamine (ZH_2, 0.5 mmol), or triamine (ZH_3 0.33

mmol), was treated with 1.1 mmol of HOBt, 1.1 mmol of DIEA, and 2.1 mmol of EDCI in 3–6 mL of CH_2Cl_2 or DMF. [Alternatively, 1 mmol of the pentafluorophenyl ester or N-hydroxysuccinimide ester of **10a** was mixed with the appropriate portion of amine (ZH), diamine (ZH_2), or triamine (ZH_3) in 3–6 mL of DMF.] The solution was stirred at room temperature under N_2 for 12–24 h, and EtOAc was added. The organic solution was washed with 5% aqueous citric acid, water, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated. The crude product was optionally purified by MPLC to afford amide **10b**. Compound **10b** was stirred in 30–80% TFA/CH_2Cl_2 for 1-3 h. The reaction mixture was concentrated to provide the TFA salt which was dissolved in CH_2Cl_2 or EtOAc and washed with aqueous NaOH or Na_2CO_3, dried over Na_2SO_4, and concentrated to give **10c** as the free base.

Scheme 10, Step B

A solution of 1 mmol of amino acid ester **10c** (n = 1), 2.5–3 mmol of the appropriately substituted chloride or bromide **2c**, and 2.5–3 mmol of an appropriate base such as DIEA, Na_2CO_3, or Cs_2CO_3 in 3–5 mL of DMF was heated at 50–100 °C under N_2 for 18–24 h. (For examples of **10c** where n = 2 or 3, the amounts of **2c** and base were increased by two- or three-fold, respectively.) The reaction mixture was cooled and partitioned between water and EtOAc. The organic layer was washed three times with water and once with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by MPLC to give pure amide **10d**.

Alternatively, a solution of 1 mmol of amino acid ester **10c** (n = 1), 2.5–3 mmol of the appropriately substituted aldehyde **2a**, and 2.5–3 mmol of borane-pyridine complex in 3–5 mL of DMF or EtOH was stirred at room temperature under N_2 for 3–5 days. . (For examples of **10c** where n = 2 or 3, the amounts of **2c** and borane-pyridine complex were increased by two- or three-fold, respectively.) The mixture was concentrated to dryness and was partitioned between water and

CH_2Cl_2, washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by MPLC to give pure amide **10d**.

Scheme 10, Step C

For examples of **10d** where R^1 = $CH_2CH_2CO_2$-t-Bu or CH_2CO_2-t-Bu, **10d** was stirred in 30–80% TFA/CH_2Cl_2 for 1-24 h. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give acid **10e**.

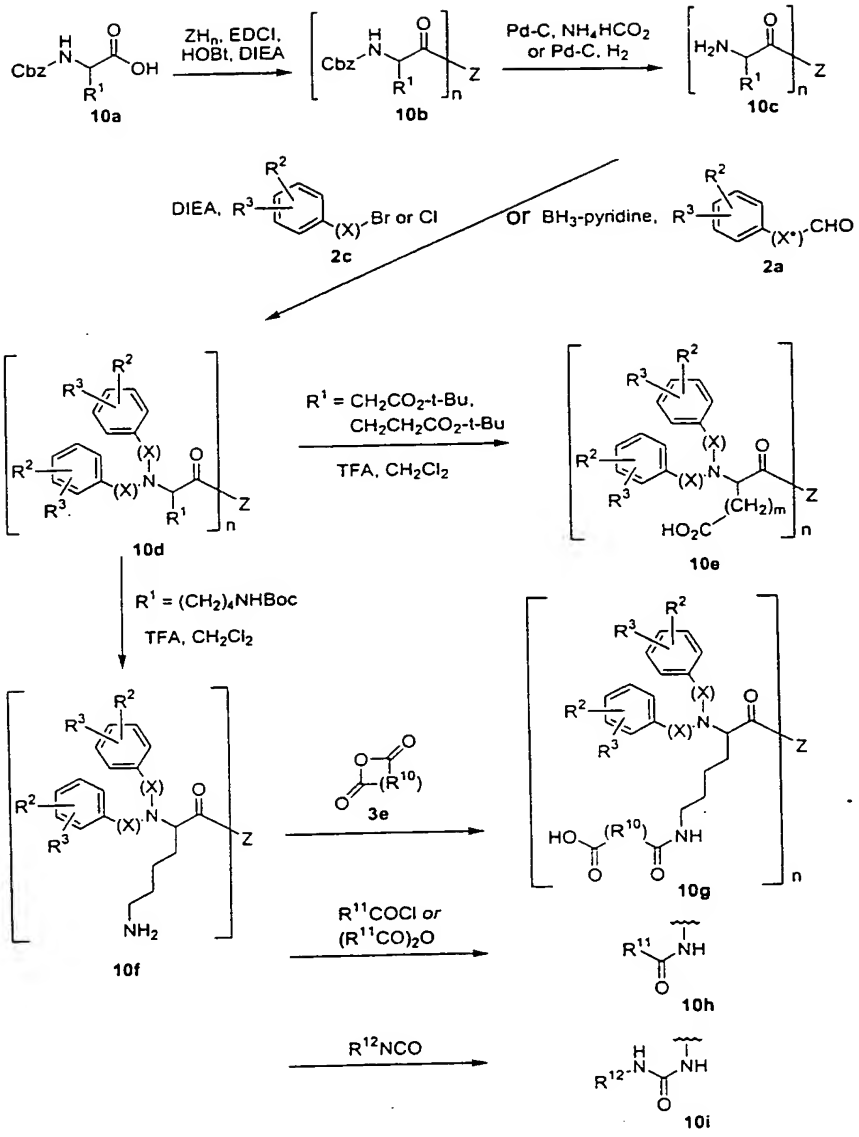
Scheme 10, Step D

For examples of **10d** where R^1 is equal to $(CH_2)_4NHBoc$, **10d** was stirred in 30–80% TFA/CH_2Cl_2 for 1-24 h. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give amine **10f** as the TFA salt which was optionally dissolved in CH_2Cl_2 or EtOAc, washed with aqueous NaOH or Na_2CO_3, dried over Na_2SO_4, and concentrated to give **10f** as the free base.

Scheme 10, Step E

A solution of 1 mmol of **10f**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted cyclic anhydride **3e** was stirred in CH_2Cl_2 or DMF under N_2 overnight. The resulting mixture was diluted with CH_2Cl_2 or EtOAc and washed with aqueous HCl, water, and brine, was dried over Na_2SO_4, and concentrated to provide acid **10g**. Alternatively, 1 mmol of **10f**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted carboxylic acid anhydride $(R^{11}CO)_2O$ or acid chloride $R^{11}COCl$ was stirred in CH_2Cl_2 or DMF under N_2 overnight and worked up as above to provide **10h**. Alternatively, 1 mmol of **8e**, 1–4 mmol of an appropriate base such as DIEA, and 1–2 mmol of the appropriately substituted isocyanate $R^{12}NCO$ was stirred in CH_2Cl_2 or DMF under N_2 overnight and worked up as above to provide **10i**.

Scheme 10.


ZHn, EDCI, HOBt, DIEA
Pd-C, NH4HCO2 or Pd-C, H2
10a
10b
10c
DIEA,
Br or Cl
2c
or BH3-pyridine,
CHO
2a
10d
R1 = CH2CO2-t-Bu, CH2CH2CO2-t-Bu
TFA, CH2Cl2
10e
R1 = (CH2)4NHBoc
TFA, CH2Cl2
10f
3e
10g
R11COCl or (R11CO)2O
10h
R12NCO
10i

General Procedure For The Solid-Phase Synthesis Of Nα,Nα-Bis-Cinnamyl Amino Acids and Nα-Cinnamyl Amino Acids

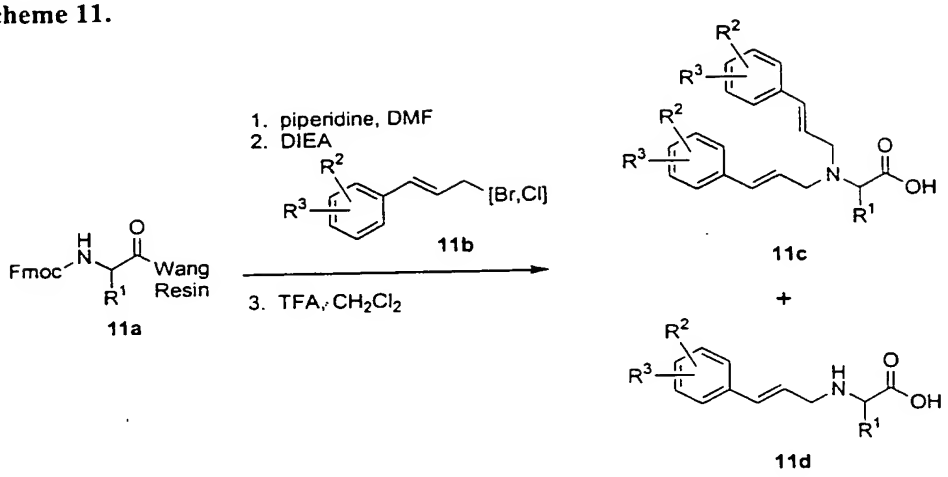
Scheme 11

An equivalent of an N-Fmoc-protected amino acid **11a** which is bound to a polystyrene resin such as Wang resin is suspended in a suitable solvent such as DMF. This solvent is removed and the nitrogen protecting group (Fmoc) is removed by stirring the resin bound amino acid with an organic base, such as piperidine, and an addition portion of the solvent. After filtration and washing with solvent, the resin is suspended in an appropriate solvent such as DMF. A solution of about 2–3 equivalents of an appropriately substituted halide **11b** and a suitable base such DIEA is added to the resin bound amino acid and this mixture is shaken for 18-36 h. The resulting mixture is washed with several portions of a suitable solvent and is suspended and shaken in an acidic solution, such as 50% TFA/CH_2Cl_2, over several hours to cleave the acid from the resin to give a mixture of the Nα,Nα-bis-cinnamyl amino acid **11c** and the Nα-cinnamyl amino acid **11d**.

By varying the resin bound amino acid 11a, one may obtain many of the compounds of the invention. The following resin bound amino acids may be used in Scheme 11: alanine, N-g-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)arginine, β-(4-methyltrityl)asparagine, aspartic acid (β-t-butyl ester), S-(trityl)cysteine, γ-(4-methyltrityl)glutamine, glutamic acid (β-t-butyl ester), glycine, N-imidazolyl-(trityl)histidine, isoleucine, leucine, N-ε-(2-chlorobenzyloxycarbonyl)lysine, N-ε-(t-butoxycarbonyl)lysine, methionine, phenylalanine, proline, O-(t-butyl)serine, O-(t-butyl)threonine, N-indolyl-(t-butoxycarbonyl)tryptophan, O-(t-butyl)tyrosine, valine, β-alanine, α-aminoadipic acid, 4-aminobutanoic acid,, 6-aminohexanoic acid, α-aminosuberic acid, 5-aminopentanoic acid, p-aminophenylalanine, α-aminopimelic acid γ-carboxyglutamic acid, p-carboxyphenylalanine, carnitine,

citrulline, α,β-diaminopropionic acid, α,γ-diaminobutyric acid, homocitrulline, homoserine, and statine.

Scheme 11.



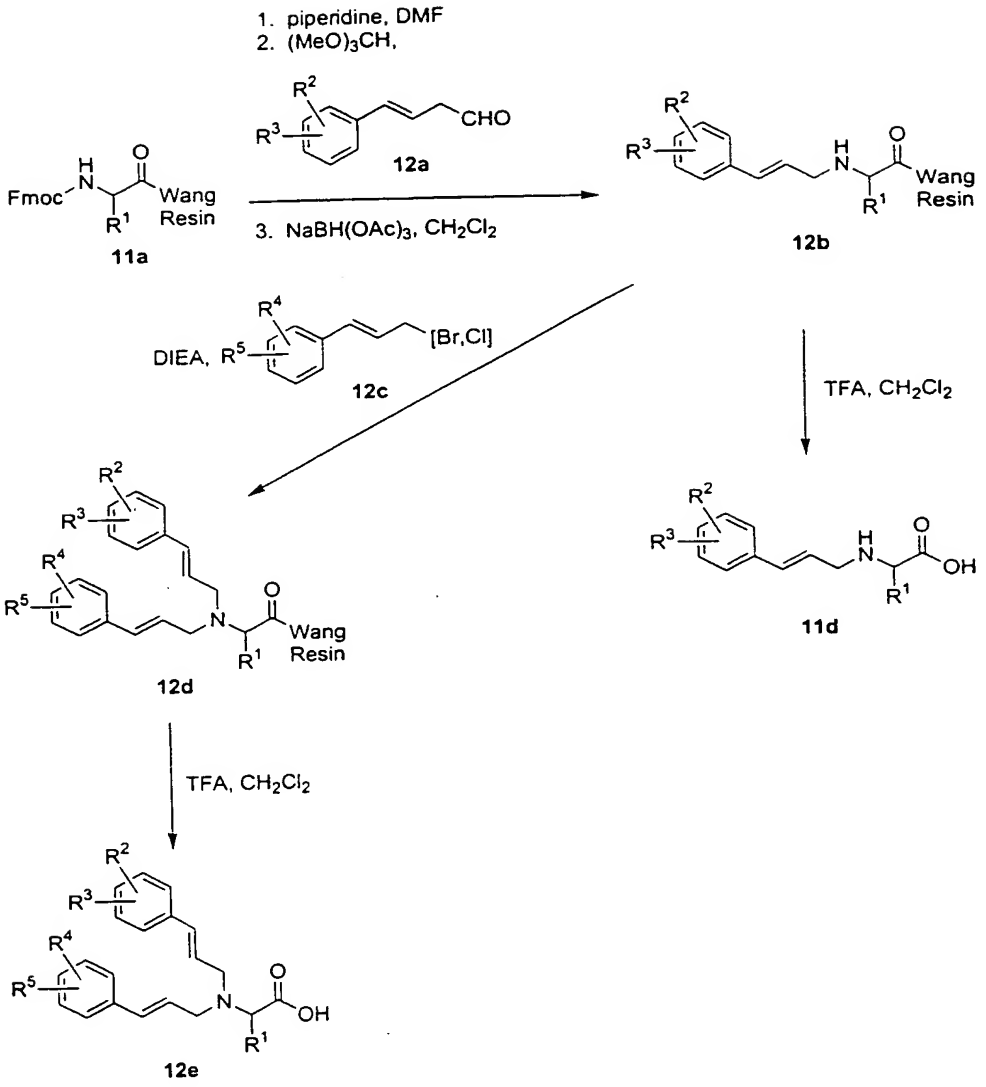
Scheme 12, Step A

An equivalent of an N-Fmoc-protected amino acid which is bound to a resin **11a** is suspended in a suitable solvent such as DMF. This solvent is removed and the nitrogen protecting group (Fmoc) is removed by stirring the resin bound amino acid with an organic base, such as piperidine, and an addition portion of the solvent. After filtration and washing with solvent, the resin is suspended in an appropriate solvent such as trimethyl orthoformate (TMOF), an appropriately substituted aldehyde **12a** (5 equivalents) is added, and the mixture is shaken under N_2 overnight. This mixture is treated with a suspension of $NaBH(OAc)_3$ (5 equivalents) in CH_2Cl_2 and shaken under N_2 overnight. After filtration and washing with a suitable solvent, the resulting product, resin bound Nα-monosubstituted amino acid **12b**, is suspended and shaken in an acidic solution, such as 50% TFA/CH_2Cl_2, over several hours to cleave the acid from the resin to give the Nα-cinnamyl amino acid **11d**.

Scheme 12, Step B

The resin **12b** is suspended in an appropriate solvent such as DMF and is filtered. The appropriately substituted halide **12c** and an appropriate base such as DIEA are added with some additional solvent and the mixture is shaken under N_2 for 18–36 h. The resin bound Nα,Nα-cinnamyl amino acid **12d** is isolated from the suspension and the resin is cleaved with an acidic solution as described above to give the free acid **12e**.

Scheme 12.

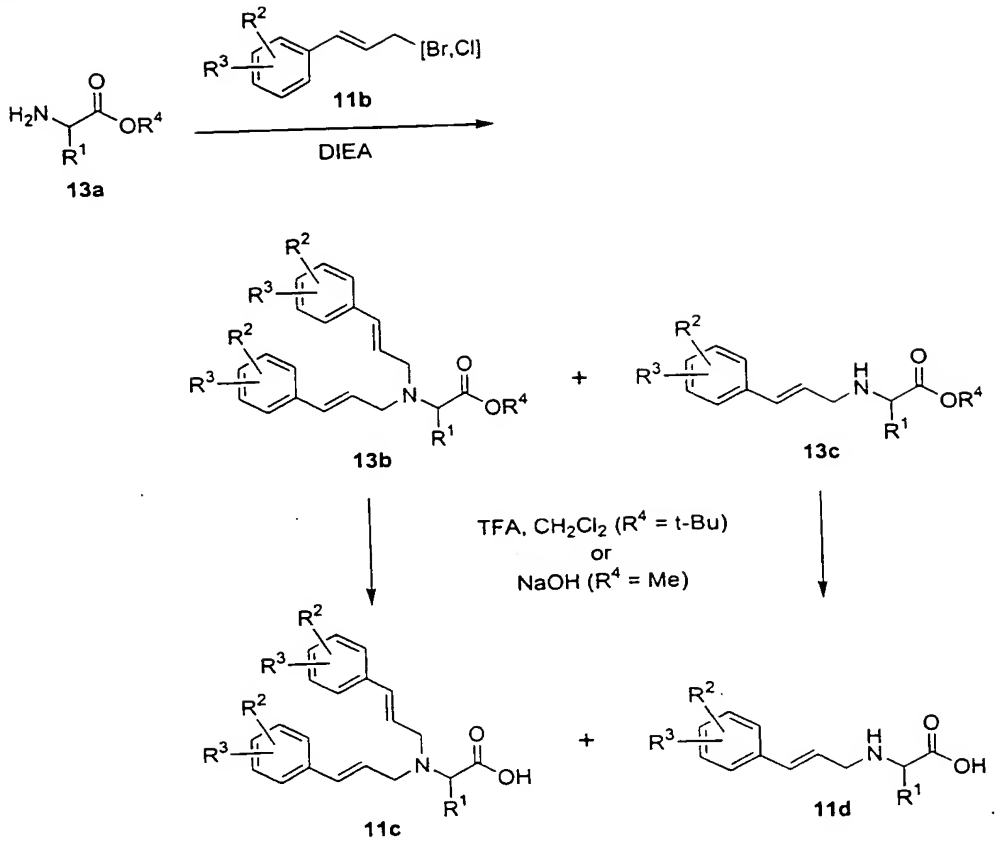

1. piperidine, DMF
2. (MeO)3CH,
3. NaBH(OAc)3, CH2Cl2
Fmoc
Wang Resin
11a
12a
12b
12c
DIEA,
[Br,Cl]
TFA, CH2Cl2
11d
12d
12e

General Procedure For The Solution-Phase Synthesis Of Nα,Nα-Bis-Cinnamyl Amino Acids and Nα-Cinnamyl Amino Acids

Scheme 13

A solution of of amino acid ester **13a**, an appropriately substituted halide **11b**, and an appropriate base such as DIEA, Na_2CO_3, or Cs_2CO_3 in a suitable solvent, such as DMF, is heated at 50–100 °C under N_2 overnight, or until the starting material is exhausted, to give a mixture of the Nα,Nα-bis-cinnamyl amino acid ester **13b** and Nα-cinnamyl amino acid ester **13c**. If the side chain of R^1 contains an acid-cleavable protecting group such as t-butylcarbamate, t-butyl ester, or t-butyl ether, those groups may be cleaved by treatment with an acidic solution such as 30–80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc. For examples of **13b** and **13c** where the ester group R^4 is a primary alkyl group such as methyl or ethyl, esters **13b** and **13c** may be independently converted to the corresponding acids **11c** and **11d** by hydrolysis with an appropriate base such as aqueous NaOH, KOH, or LiOH. For examples of **13b** and **13c** where the ester group R^4 is an acid-cleavable group such as t-butyl, esters **13b** and **13c** may be independently converted to the corresponding acids **11c** and **11d** by treatment with an acidic solution such as 30-80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc.

Scheme 13.



Scheme 14, Step A

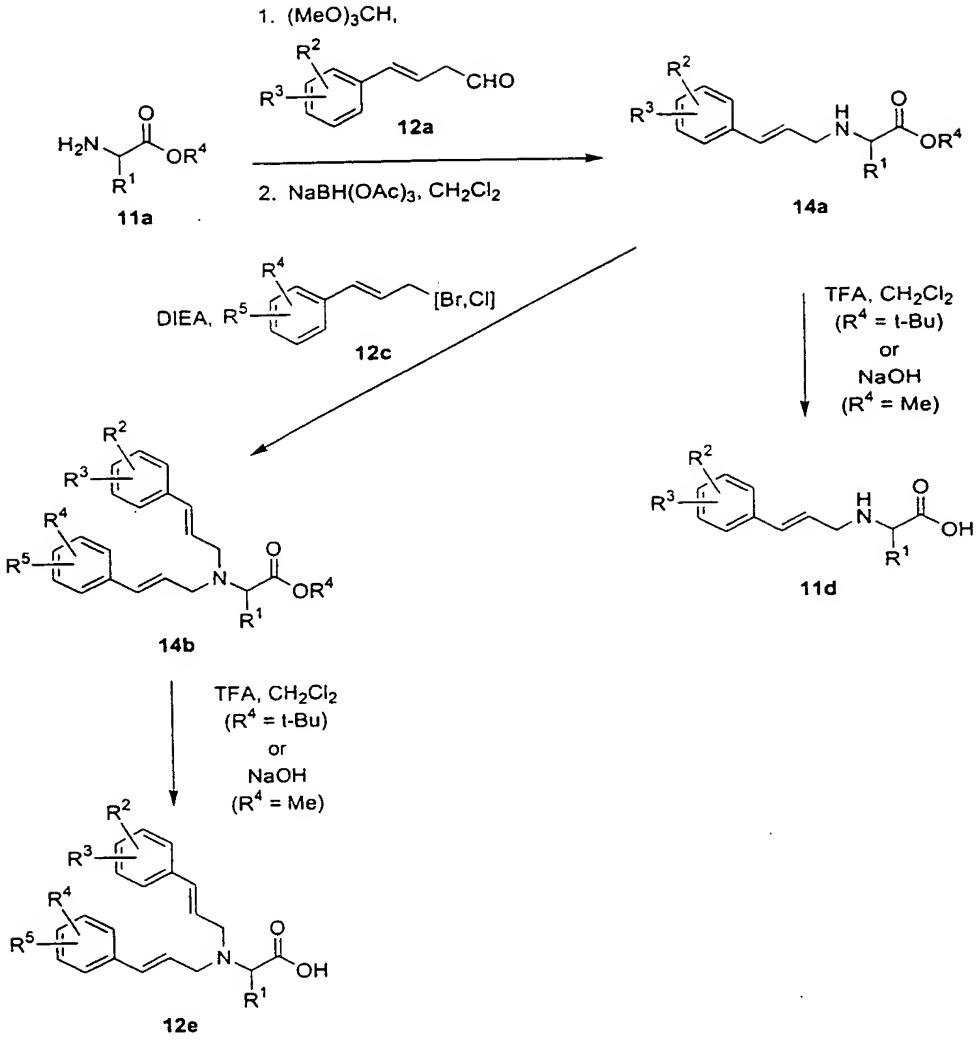
A solution of 1 mmol of amino acid ester and 1–1.5 mmol of the appropriately substituted aldehyde **12a** in 3–5 mL of TMOF was stirred at room temperature under N_2 overnight. The solution was concentrated and used directly for the next reaction; optionally, the solution was partitioned between EtOAc and water, washed with brine, dried over Na_2SO_4, and concentrated to give crude product, which was purified by MPLC to give mono-substituted product **14a**. For examples of **14a** wherein the side chain R^1 contained an acid-cleavable protecting

group such as t-butylcarbamate, t-butyl ester, or t-butyl ether, 8c was treated with an acidic solution such as 30–80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give the deprotected form of **14a**. For examples of **14a** where the ester group R^4 is a primary alkyl group such as methyl or ethyl, esters **14a** may be converted to the corresponding acids **11d** by hydrolysis with an appropriate base such as aqueous NaOH, KOH, or LiOH. For examples of **14a** where the ester group R^4 is an acid-cleavable group such as t-butyl, esters **14a** may be converted to the corresponding acids **11d** by treatment with an acidic solution such as 30-80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc.

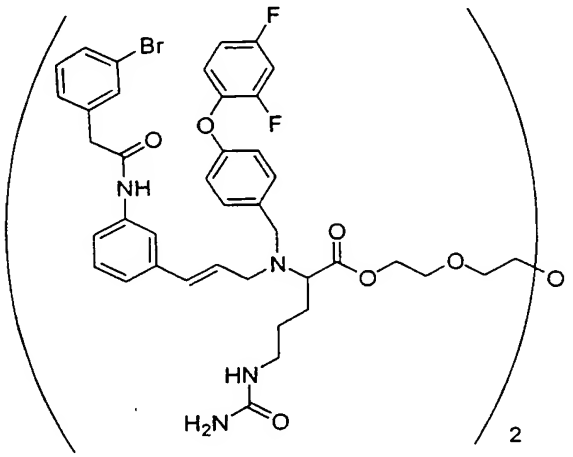
Scheme 14, Step B

Amino ester **14a** was dissolved in DMF, combined with 1.1–1.5 mmol of the appropriately substituted chloride or bromide **12c**, and heated at 50–100 °C overnight. The reaction mixture was cooled and partitioned between water and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by MPLC to give pure **14b**. For examples of **14b** wherein the side chain R^1 contained an acid-cleavable protecting group such as t-butylcarbamate, t-butyl ester, or t-butyl ether, 8c was treated with an acidic solution such as 30–80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc. The reaction mixture was concentrated and optionally dissolved in HOAc and freeze-dried to give the deprotected form of **14b**. For examples of **14b** where the ester group R^4 is a primary alkyl group such as methyl or ethyl, esters **14b** may be converted to the corresponding acids **12e** by hydrolysis with an appropriate base such as aqueous NaOH, KOH, or LiOH. For examples of **14b** where the ester group R^4 is an acid-cleavable group such as t-butyl, esters **14b** may be converted to the corresponding acids **12e** by treatment with an acidic solution such as 30-80% TFA/CH_2Cl_2 or 2–4N HCl in EtOAc.

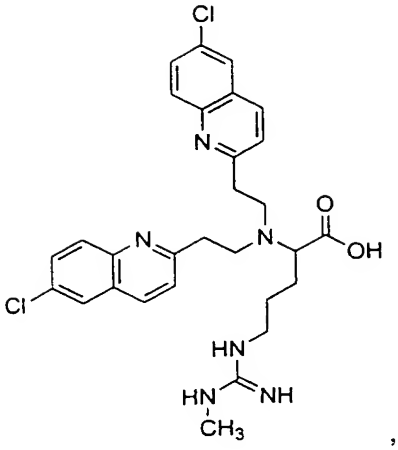
Scheme 14.


1. (MeO)3CH,
12a
2. NaBH(OAc)3, CH2Cl2
11a
14a
DIEA,
[Br,Cl]
12c
TFA, CH2Cl2
(R4 = t-Bu)
or
NaOH
(R4 = Me)
11d
14b
TFA, CH2Cl2
(R4 = t-Bu)
or
NaOH
(R4 = Me)
12e

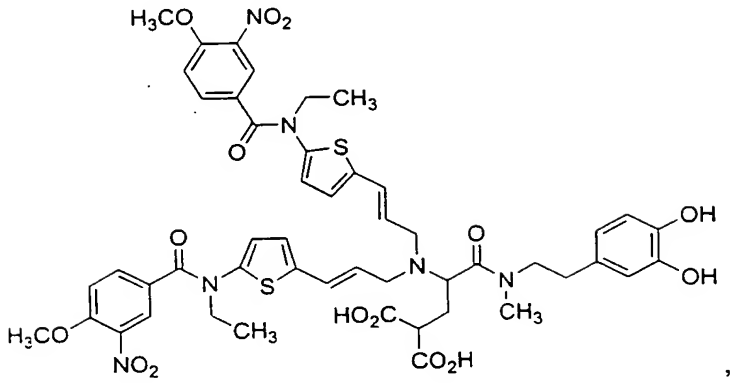
Although the claimed compounds are useful as competitive binders to the EPO receptor, some compounds are more active than others and are either preferred or particularly preferred.



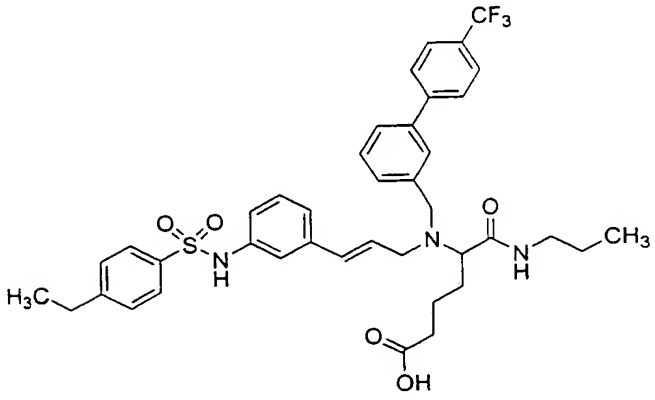
,



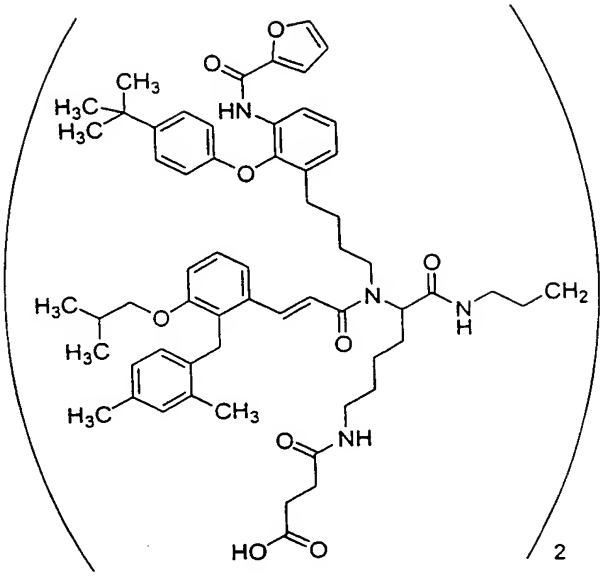
,



,



, and



The particularly preferred "R^1 "s are the side chain of lysine, ornithine, arginine, aspartic acid, glutamic acid, glutamine, cysteine, methionine, serine, and threonine.

The particularly preferred "R^2 and R^3" s are phenoxy, substituted phenoxy, benzyloxy, and substituted benzyloxy.

The particularly preferred "R^4 and R^5" s are phenoxy, substituted phenoxy, benzyloxy, and substituted benzyloxy.

The particularly preferred "W" is -CH=CH-

The particularly preferred "Q" is -CH=CH-

The particularly preferred "X" are $C_{1\text{-}5}$alkenyl and CH_2.

The particularly preferred "Y" are $C_{1\text{-}5}$alkenyl and CH_2.

The particularly preferred "n" are 1 and 2.

The particularly preferred "Z" are hydroxy, methoxy, phenethylamino, substituted phenethylamino, and $-NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH-$.

Pharmaceutically useful compositions the compounds of the present invention, may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the compound of the present invention.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders in which modulation of EPO receptor-related activity is indicated. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, transdermal, oral and parenteral.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds disclosed herein may be used alone at appropriate dosages defined by routine testing in order to obtain optimal inhibition of the EPO receptor or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, transdermal, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds according to this invention as the active ingredient for use in the

modulation of EPO receptors can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds or modulators can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by transdermal delivery or injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, transdermal, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. The compounds of the present invention may be delivered by a wide variety of mechanisms, including but not limited to, transdermal delivery, or injection by needle or needle-less injection means. An effective but non-toxic amount of the compound desired can be employed as an EPO receptor modulating agent.

The daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per patient, per day. For oral administration, the compositions are preferably provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per day. The dosages of the EPO receptor modulators are adjusted when combined to achieve desired effects. On the other hand, dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone.

Advantageously, compounds or modulators of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds or

modulators for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds or modulators of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

In the methods of the present invention, the compounds or modulators herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically

acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

For liquid forms the active drug component can be combined in suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methyl-cellulose and the like. Other dispersing agents which may be employed include glycerin and the like. For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable preservatives are employed when intravenous administration is desired.

Topical preparations containing the active drug component can be admixed with a variety of carrier materials well known in the art, such as, e.g., alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form, e.g., alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations.

The compounds or modulators of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds or modulators of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-amidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyl-eneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds or modulators of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels, and other suitable polymers known to those skilled in the art.

For oral administration, the compounds or modulators may be administered in capsule, tablet, or bolus form or alternatively they can be mixed in the animals feed. The capsules, tablets, and boluses are comprised of the active ingredient in combination with an appropriate carrier vehicle such as starch, talc, magnesium stearate, or di-calcium phosphate. These unit dosage forms are prepared by intimately mixing the active ingredient with suitable finely-powdered inert ingredients including diluents, fillers, disintegrating agents, and/or binders such that a uniform mixture is obtained. An inert ingredient is one that will not react with the compounds or modulators and which is non-toxic to the animal being treated. Suitable inert ingredients include starch, lactose, talc, magnesium stearate, vegetable gums and oils, and the like. These formulations may contain a widely variable amount of the active and inactive ingredients depending on numerous factors such as the size and type of the animal species to be treated and the type and severity of the infection. The active ingredient may also be administered as an additive to the feed by simply mixing the compound with the feedstuff or by applying the compound to

the surface of the feed. Alternatively the active ingredient may be mixed with an inert carrier and the resulting composition may then either be mixed with the feed or fed directly to the animal. Suitable inert carriers include corn meal, citrus meal, fermentation residues, soya grits, dried grains and the like. The active ingredients are intimately mixed with these inert carriers by grinding, stirring, milling, or tumbling such that the final composition contains from 0.001 to 5% by weight of the active ingredient.

The compounds or modulators may alternatively be administered parenterally via injection of a formulation consisting of the active ingredient dissolved in an inert liquid carrier. Injection may be either intramuscular, intraruminal, intratracheal, or subcutaneous, either by needle or needle-less means. The injectable formulation consists of the active ingredient mixed with an appropriate inert liquid carrier. Acceptable liquid carriers include the vegetable oils such as peanut oil, cotton seed oil, sesame oil and the like as well as organic solvents such as solketal, glycerol formal and the like. As an alternative, aqueous parenteral formulations may also be used. The vegetable oils are the preferred liquid carriers. The formulations are prepared by dissolving or suspending the active ingredient in the liquid carrier such that the final formulation contains from 0.005 to 10% by weight of the active ingredient.

Topical application of the compounds or modulators is possible through the use of a liquid drench or a shampoo containing the instant compounds or modulators as an aqueous solution or suspension. These formulations generally contain a suspending agent such as bentonite and normally will also contain an antifoaming agent. Formulations containing from 0.005 to 10% by weight of the active ingredient are acceptable. Preferred formulations are those containing from 0.01 to 5% by weight of the instant compounds or modulators.

The compounds of Formula I may be used in pharmaceutical compositions to treat patients (humans and other mammals) with disorders or conditions associated with the production of erythropoietin or modulated by the EPO receptor. The compounds can be administered in the manner of the commercially available product or by any oral or parenteral route (including but not limited to, intravenous, intraperitoneal, intramuscular, subcutaneous, dermal patch), where the preferred route is by injection. When the method of administration is intravenous infusion, compound of Formula I may be administered in a dose range of about 0.01 to 1 mg/kg/min. For oral administration, the dose range is about 0.1 to 100 mg/kg.

The pharmaceutical compositions can be prepared using conventional pharmaceutical excipients and compounding techniques. Oral dosage forms may be used and are elixirs, syrups, capsules, tablets and the like. Where the typical solid carrier is an inert substance such as lactose, starch, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, mannitol and the like; and typical liquid oral excipients include ethanol, glycerol, water and the like. All excipients may be mixed as needed with disintegrants, diluents, granulating agents, lubricants, binders and the like using conventional techniques known to those skilled in the art of preparing dosage forms. Parenteral dosage forms may be prepared using water or another sterile carrier.

Typically the compounds of Formula I are isolated as the free base, however when possible pharmaceutically acceptable salts can be prepared. Examples of such salts include hydrobromic, hydroiodic, hydrochloric, perchloric, sulfuric, maleic, fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroethanesulfonic, benzenesulfonic, oxalic, pamoic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic and saccharic.

In order to illustrate the invention the following examples are included. These examples do not limit the invention. They are only meant to suggest a method of practicing the invention. Those knowledgeable in chemical synthesis and

the treatment of EPO related disorders may find other methods of practicing the invention. However those methods are deemed to be within the scope of this invention.

BIOLOGICAL EXAMPLES

The compounds of the invention were evaluated for the ability to compete with EPO in the following immobilized EPO receptor preparation (EBP-Ig, EPO binding protein-Ig).

EBP-Ig fusion protein (as disclosed in WO97/27219 which is herein incorporated by reference) was purified by affinity chromatography from the conditioned media of NSO cells engineered to express a recombinant gene construct which functionally joined the N-terminal 225 amino acids of the human EPO receptor and an Ig heavy chain as described herein. The interaction of biotin and streptavidin is frequently employed to capture and effectively immobilize reagents useful in assay protocols and has been employed here as a simple method to capture and immobilize EBP-Ig. EBP-Ig is initially randomly modified with an amine reactive derivative of biotin to produce biotinylated-EBP-Ig. Use of streptavidin coated plates allows the capture of the biotinylated EBP-Ig on the surface of a scintillant impregnated coated well (Flash plates, NEN-DuPont). Upon binding of [^{125}I]EPO to the ligand binding domain, specific distance requirements are satisfied and the scintillant is induced to emit light in response to the energy emitted by the radioligand. Unbound radioligand does not produce a measurable signal because the energy from the radioactive decay is too distant from the scintillant. The amount of light produced was quantified to estimate the amount of ligand binding. The specific assay format was suitable for the multi-well plate capacity of a Packard TopCount Microplate Scintillation counter. Compounds which were capable of

reducing the amount of detected signal through competitive binding with the radioligand were identified.

Biotinylated EBP-Ig was prepared as follows. EBP-Ig (3 mL, OD_{280} 2.9) was exchanged into 50 mM sodium bicarbonate, pH 8.5 using a Centriprep 10 ultrafiltration device. The final volume of the exchanged protein was 1.2 mL (OD_{280} 2.6, representing about 2 mg total protein). 10 µL of a 4 mg/ml solution of NHS-LC-Biotin (Pierce) was added and the reaction mixture placed on ice in the dark for two hours. Unreacted biotin was removed by exchange of the reaction buffer into PBS in a Centriprep 10 device and the protein reagent aliquoted and stored at -70 °C.

Each individual binding well (200 µL) contained final concentrations of 1 µg/mL of biotinylated EBP-Ig, 0.5 nM of [^{125}I]EPO (NEN Research Products, Boston, 100 µCi/µg) and 0–500 µM of test compound (from a 10–50 mM stock in 100% DMSO). All wells were adjusted to a final DMSO concentration of 5%. All assay points were performed in triplicate and with each experiment a standard curve for unlabelled EPO was performed at final concentration of 2000, 62, 15, 8, 4, and 0 nM. After all additions were made, the plate was covered with an adhesive top seal and placed in the dark at room temperature overnight. The next day all liquid was aspirated from the wells to limit analyte dependent quench of the signal, and the plates were counted on a Packard TOPCOUNT Microplate Scintillation Counter. Non-specific binding (NSB) was calculated as the mean CPM of the 2000 nM EPO wells and total binding (TB) as the mean of the wells with no added unlabelled EPO. Corrected total binding (CTB) was calculated as: TB – NSB = CTB. The concentration of test compound which reduced CTB to 50% was reported as the IC_{50}. Typically the IC_{50} value for unlabelled EPO was ca. 2–7 nM and EMP1 was 0.1 µM. Table 1 lists the average % inhibition, and if determined the IC_{50} and IC_{30} values for compounds of Formula I, where the compound numbers refer to the compounds in the tables accompanying the preparative examples.

Table 1. Inhibition of EPO binding to EBP-Ig

cpd	% inh @ 50 μM	IC_{30} μM*	IC_{50}, μM*
11	70	nd	nd
12	59	nd	nd
14	30	nd	nd
15	48	nd	nd
77	52	30	40
82	32	nd	nd
86	37	nd	nd
100	34	nd	nd
101	32	nd	nd
104	78	10	30
105	70	25	35
107	78	30	42
108	81	23	36
110	54	6	10
112	59	2	10
114	37	10	nd
115	35	nd	nd
116	32	nd	nd
117	34	nd	nd
118	36	2	10
119	34	nd	nd
120	35	nd	nd
121	45	6	nd
137	60	5	30
139	46	2	10
178	36	nd	nd
179	30	nd	nd

183	36	nd	nd
184	53	10	nd
203	37	50	nd
211	62	20	65
220	45	30	50
221	48	10	80
222	56	5	nd
224	51	25	50
227	48	20	50
230	42	nd	nd
231	36	nd	nd
235	49	20	50
237	55	30	70
238	39	nd	nd
239	46	8	50
243	75	2	18
244	66	1	28
246	79	10	75
247	47	7	18
248	56	7	20
249	72	7	10
250	78	7	20
251	49	10	45
261	51	1.5	2
262	93	1	1.5
263	88	1	1.5
264	89	1.5	8
265	65	1	6
266	82	1	4
267	83	2	6

268	40	nd	nd
269	55	8	85
270	56	7	100
271	77	2	7
272	78	5	10
285	41	nd	nd
286	46	35	65
287	36	nd	nd
300	57	35	145
305	48	35	225
312	45	10	85
321	42	45	nd
363	33	35	220
366	38	65	nd
368	40	90	nd

*nd = not determined

PREPARATIVE EXAMPLES

Unless otherwise noted, materials used in the examples were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured in the indicated solvent with tetramethylsilane (TMS) as the internal standard using a Bruker AC-300 NMR spectrometer. NMR chemical shifts are expressed in parts per million (ppm) downfield from internal TMS using the d scale. ^{1}H NMR data are tabulated in order: multiplicity, (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant in Hertz). Electrospray (ES) mass spectra

(MS) were determined on a Hewlett Packard Series 1090 LCMS Engine. Elemental analyses were performed by Quantitative Technologies, Inc. (QTI), PO Box 470, Salem Industrial Park, Bldg #5, Whitehouse, NJ 08888-0470. Analytical thin layer chromatography (TLC) was done with Merck Silica Gel 60 F_{254} plates (250 micron). Medium pressure liquid chromatography (MPLC) was done with Merck Silica Gel 60 (230-400 mesh).

Example 1

N,N-bis(3-Phenoxycinnamyl)Glu(O-t-Bu)-OMe (cpd 96) and N-(3-phenoxycinnamyl)Glu(O-t-Bu)-OMe (cpd 334)

A solution of 500 mg (1.97 mmol) of H-Glu(O-t-Bu)OMe•HCl, 997 mg (3.45 mmol) of 3-phenoxycinnamyl bromide (Jackson, W. P.; Islip, P. J.; Kneen, G.; Pugh, A.; Wates, P. J. *J.Med.Chem.* **31** *1988*; 499-500), and 0.89 mL (5.1 mmol, 660 mg) of DIEA in 5 mL of DMF was stirred under N_2 at room temperature for 40 h. The mixture was partitioned between EtOAc and water and the organic layer was washed with water and brine. After drying over Na_2SO_4, the organic solution was concentrated to give 1.24 g of orange oil. The crude residue was purified by MPLC using a solvent gradient ranging from 10–30% EtOAc/hexanes to give two products. The less polar product (**cpd 96**, 235 mg, 19% based on starting amino acid), was isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.39 (s, 9H), 2.0 (m, 2H), 2.33 (dt, 2H, J = 2, 7 Hz), 3.24 (dd, 2H, J = 8, 15 Hz), 3.5, (m, 3H), 3.69 (s, 3H), 6.13 (m, 2H), 6.47 (d, 2H, J = 16 Hz), 6.86 (dd, 2H, J = 1.5, 8 Hz), 7.0–7.4 (complex, 16H); MS (ES+) m/z 634 (MH+).

The more polar product (**cpd 334**, 422 mg, 50% based on starting amino acid) was isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.42 (s, 9H), 1.9 (m, 2H), 2.35 (t, 2H, J = 7.5 Hz), 3.2–3.4 (complex, 3H), 3.71 (s, 3H), 6.17 (dt, 1H, J = 16, 6 Hz), 6.46 (d, 1H, J = 16 Hz), 6.87 (dd, 1H, J = 1.5, 8 Hz), 7.01 (m, 3H), 7.10 (t, 2H, J = 7.5 Hz), 7.2–7.4 (complex, 3H); MS (ES+) m/z 426 (MH+). Anal.

Calcd for $C_{25}H_{31}NO_5$: C, 70.57; H, 7.34; N, 3.29. Found: C, 70.29; H, 7.14; N, 3.08.

Example 2

N-(3-Phenoxycinnamyl)Glu-OMe (cpd 325)

A solution of 95 mg (0.22 mmol) of N-(3-phenoxycinnamyl)Glu(O-t-Bu)-OMe (**cpd 334**) in 3 mL of 50% TFA/CH_2Cl_2 was stirred for 2 h at room temperature. The mixture was concentrated and the residue was dissolve in acetic acid and freeze-dried to give 117 mg of N-(3-phenoxycinnamyl)Glu-OMe (**cpd 325**)as an off-white solid; ^{1}H NMR (CD_3OD, 300 MHz) 2.3–2.7 (complex, 4H), 3.78 (s, 3H), 3.81 (d, 2H, J = 7 Hz), 4.09 (t, 1H, J = 5 Hz), 6.17 (dt, 1H, J = 16, 7 Hz), 6.55 (d, 1H, J = 16 Hz), 6.9 (m, 4H), 7.11 (t, 2H, J = 7.5 Hz), 7.3 (m, 4H); MS (ES+) m/z 370 (MH+), 209. Anal. Calcd for $C_{21}H_{23}NO_5 \cdot C_2HF_3O_2$: C, 57.14; H, 5.00; N, 2.90. Found: C, 57.07; H, 5.02; N, 2.73.

Example 3

N,N-bis(3-Phenoxycinnamyl)Asp(O-t-Bu)-O-t-Bu (cpd 106)

A solution of 1.00 g (3.55 mmol) of Asp(O-t-Bu)-O-t-Bu•HCl, 2.05 g (7.1 mmol) of 3-phenoxycinnamyl bromide, and 1.86 mL (10.7 mmol, 1.38 g) of DIEA in 15 mL of DMF was heated under N_2 at 60 °C overnight. Additional 3-phenoxycinnamyl bromide (1.0 g, 3.4 mmol) and DIEA (0.95 mL, 0.705 g, 5.4 mmol) were added and heating was continued for an additional 14 h. The mixture was cooled and partitioned between EtOAc and water. The organic layer was washed twice with water, once with brine, and was dried over Na_2SO_4. The solution was concentrated to give 3.5 g of an amber oil which was purified by MPLC using a solvent gradient ranging from 2.5–3% EtOAc/hexanes to afford 1.21 g of **cpd 106** as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.41 (s, 9H), 1.48 (s, 9H), 2.49 (dd, 1H, J = 7.5, 15.5 Hz), 2.70 (dd, 1H, J =7.5, 15.5 Hz), 3.26 (dd, 2H, J = 7.5, 14.5 Hz),

3.47 (dd, 2H, J = 4, 14.5 Hz), 3.88 (t, 1H, J = 7.5), 6.13 (m, 2H), 6.48 (d, 2H, J = 16 Hz), 6.86 (dd, 2H, J = 2, 8 Hz), 7.0 (m, 6H), 7.1 (m, 4H), 7.2–7.4 (complex, 6H); MS (ES+) m/z 662 (MH+).

Example 4

N,N-bis(3-Phenoxycinnamyl)Asp-OH (cpd 107)

A solution of 1.14 g (1.62 mmol) of **cpd 106** in 16 mL of 50% TFA/CH_2Cl_2 was stirred at room temperature for 24 h. The solution was concentrated and pumped to give 1.37 g (~100%) **cpd 107** as an amber oil; ^{1}H NMR (CD_3OD, 300 MHz) 3.1 (m, 2H), 4.0 (dd, 2H, J = 8, 14 Hz), 4.27 (dd, 2H, J = 8, 16 Hz), 4.70 (t, 1H, J = 4 Hz), 6.38 (2H, dt, J = 16, 8 Hz), 6.7–7.4 (complex, 20H); MS (ES-) m/z 562 ([M-H]+).

Example 5

N,N-bis(4-Benzyloxybenzyl)Lys(Boc)-OMe (cpd 111)

and N-(4-Benzyloxybenzyl)Lys(Boc)-OMe

A solution of 594 mg (2.0 mmol) of Lys(Boc)-OMe•HCl, 524 mg (2.25 mmol) of 4-benzyloxybenzyl chloride, 75 mg (0.5 mmol), of NaI, and 0.61 mL (3.5 mol, 452 mg) of DIEA was warmed at 50–70 °C under N_2 overnight. The mixture was cooled and partioned between EtOAc and water. The organic layer was washed twice with water, once with brine, and was dried over Na_2SO_4. The organic solution was concentrated to give 0.83 g of amber oil which was purified by MPLC using a solvent gradient ranging from 15–40% EtOAc/hexanes to give two products. The less polar product (296 mg), **cpd 111**, was isolated as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.28 (m, 4H), 1.43 (s, 9H), 1.70 (m, 2H), 3.03 (m, 2H), 3.28 (t, 1H, J = 7 Hz), 3.40 (d, 2H, J = 13.5 Hz), 3.74 (s, 3H), 3.81 (d, 2H, J = 13.5 Hz), 5.05 (2, 4H), 6.92 (d, 4H, J = 8.5), 7.23 (d, 4H, J = 8.5), 7.25–7.5 (complex, 10H); MS (ES+) m/z 653 (MH+).

The more polar product (406 mg), N-(4-benzyloxybenzyl)Lys(Boc)-OMe, was isolated as a white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.4 (, 4H), 1.43 (s, 9H), 1.65 (m, 3H), 3.08 (m, 2H), 3.23 (t, 1H, J = 6.5 Hz), 3.54 (d, 1H, J = 12.5 Hz), 3.71 (s, 3H), 3.73 (d, 1H, J = 12.5 Hz), 5.05 (s, 2H), 6.92 (d, 2H, J = 8.5 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.25–7.5 (complex, 5H); MS (ES+) m/z 457 (MH+).

Example 6

N-(4-Benzyloxybenzyl)-N-(3-nitrobenzyl)Lys(Boc)-OMe (cpd 113)

A solution of 374 mg (0.82 mmol) of N-(4-Benzyloxybenzyl)Lys(Boc)-OMe, 221 mg (1.03 mmol) of 4-nitrobenzyl bromide, and 197 L (1 .13 mmol, 146 mg) of DIEA was warmed at 50–70 °C for 4 h, then at 40–50 °C overnight. After the addition of 0.2 mL of 1N aqueous HCl, the mixture was partioned between EtOAc and water. The organic layer was washed twice with water, once with brine, and was dried over Na_2SO_4. The organic solution was concentrated to give 610 mg of an amber oil which was purified by MPLC 1:3 EtOAc/hexanes to afford 436 mg (90%) **cpd 113** as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.35 (m, 4H), 1.42 (s, 9H), 1.75 (broad q, 2H, J = 8 Hz), 3.06 (broad q, 2H, J = 6 Hz), 3.28 (t, 1H, J = 7.5 Hz), 3.48 (d, 1H, J = 13.5 Hz), 3.66 (d, 1H, J = 14.5 Hz), 3.76 (s, 3H), 3.79 (d, 1H, J = 13.5 Hz), 3.97 (d, 1H, J = 14.5 Hz), 4.47 (broad s, 1H), 5.05 (s, 2H), 6.93 (d, 2H, J = 8.5 Hz), 7.22 (d, 2H, J = 8.5 Hz), 7.3–7.5 (complex, 6H), 7.65 (d, 1H, J = 7.5 Hz), 8.09 (d, 1H, J = 8 Hz), 8.22 (s, 1H); MS (ES+) m/z 592 (MH+).

Example 7

N-(3-Aminobenzyl)-N-(4-benzyloxybenzyl)Lys(Boc)-OMe

A solution of 361 mg (0.61 mmol) of **cpd 113** and 835 mg (3.7 mmol) of $SnCl_2$ dihydrate was stirred under N_2 at room temperature for 6 h. The slightly cloudy mixture was poured into 200 mL of 5% aqueous Na_2CO_3 with rapid stirring. The resulting milky suspension was extracted with three 75 mL portions of CH_2Cl_2

and the combined organic layers were washed with brine and dried over Na_2SO_4. The extracts were concentrated to give 344 mg of colorless oil which was purified by MPLC using 1:2 EtOAc/hexanes to provide 291 mg of N-(3-aminobenzyl)-N-(4-benzyloxybenzyl)Lys(Boc)-OMe as a yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.25 (m, 4H), 1.44 (s, 9H), 1.70 (m, 2H), 3.31 (dd, 1H, J = 6, 9 Hz), 3.38 (d, 1H, J = 14 Hz), 3.40 (d, 1H, J = 13.5 Hz), 3.74 (s, 3H), 3.81 (d, 1H, J = 14 Hz), 3.83 (d, 1H, J = 13.5 Hz), 4.52 (broad s, 1H), 5.05 (s, 2H), 6.50 (broad d, 1H, J = 8 Hz), 6.70 (m, 2H), 6.92 (d, 2H, J = 8.5 Hz), 7.08 (t, 1H, J = 7.5 Hz), 7.2–7.5 (complex, 7H); MS (ES+) m/z 562 (base, MH+), 506.

Example 8

N-(4-Benzyloxybenzyl)-N-(3-((2-furancarbonyl)amino)benzyl)Lys-OMe (cpd 117)

A solution of 42 mg (0.075 mmol) of N-(3-aminobenzyl)-N-(4-benzyloxybenzyl)Lys(Boc)-OMe and 12 μL (12 mg, 0.15 mmol) of pyridine in 0.5 mL of 1,2-dichloroethane was combined with 8.1 μL (11 mg, 0.083 mmol) and stirred under N_2 overnight. EtOAc (3 mL) was added and the solution was washed twice with 2 mL of water and 2 mL of saturated aqueous $NaHCO_3$. The EtOAc solution was filtered through a pad of Na_2SO_4 and concentrated to give 44 mg of N-(4-benzyloxybenzyl)-N-(3-((2-furancarbonyl)amino)benzyl)Lys(Boc)-OMe; MS (ES+) m/z 356 (MH+). The Boc-protected intermediate was stirred in 2 mL of 50% TFA/CH_2Cl_2 for 2 h and was concentrated and pumped at high vacuum to provide 66 mg of **cpd 117** as the bis-TFA salt; ^{1}H NMR (CD_3OD, 300 MHz) 1.55 (m, 2H), 1.65 (m, 2H), 2.10 (m, 2H), 2.93 (t, 2H, J = 7 Hz), 3.68 (t, 1H, J = 7 Hz), 3.78 (s, 3H), 4.20 (m, 4H), 5.09 (s, 2H), 6.66 (dd, 1H, J = 1.5, 3.5 Hz), 7.03 (d, 2H, J = 8.5 Hz), 7.1–7.6 (complex, 11H), 7.76 (m, H), 8.07 (m, 1H); MS (ES+) m/z 556 (base, MH+), 360, 197.

Example 9

N,N-bis(3-Nitrobenzyl)Asp(O-t-Bu)-O-t-Bu (cpd 62)

A solution of 0.50 mg (1.77 mmol) of Asp(O-t-Bu)-O-t-Bu•HCl, 1.17 g (5.42 mmol) of 3-nitrobenzyl bromide, and 1.25 mL (0.93 g, 7.2 mmol) of DIEA in 6 mL of DMF was stirred at room temperature under N_2 for 24 h and was heated at 70–80 °C overnight. The reaction mixture was partitioned between EtOAc and water and the organic layer was washed twice with water and once with brine. After drying over Na_2SO_4, the organic solution was concentrated to give 0.86 g of a yellow oil which was purified by MPLC using 1:9 EtOAc/hexanes to afford 0.849 g (93%) **cpd 62** as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.43 (s, 9H), 1.57 (s, 9H), 2.59 (dd, 1H, J = 7.5, 16 Hz), 2.76 (dd, 1H, J = 7, 16 Hz), 3.72 (t, 1H, J = 7.5 Hz), 3.78 (d, 2H, J = 14 Hz), 3.92 (d, 2H, J = 14 Hz), 7.47 (t, 2H, J = 8 Hz), 7.67 (d, 2H, J = 7.5 Hz), 8.09 (broad d, 2H J = 8 Hz), 8.16 (broad s, 2H); MS (ES+) m/z 538 (MNa+), 516 (base, MH+), 460, 404, 237.

Example 10

N,N-bis(3-Aminobenzyl)Asp(O-t-Bu)-O-t-Bu

A solution of 0.644 g (1.25 mmol) of **cpd 62** and 2.82 g (12.5 mmol) of $SnCl_2$•2 H_2O in 12 mL of absolute EtOH was refluxed for 1.5 h. The mixture was cooled and poured into 300 mL of 5% aqueous Na_2CO_3 with rapid stirring. The cloudy mixture was extracted with three 150 mL portions of CH_2Cl_2 and the organic extracts were washed with brine and dried over Na_2SO_4. The CH_2Cl_2 solution was concentrated to afford 210 mg (37%) of N,N-bis(3-aminobenzyl)Asp(O-t-Bu)-O-t-Bu as a cloudy yellow oil which was used without purification; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.40 (s, 9H), 1.52 (s, 9H), 2.48 (dd, 1H, J = 7, 16 Hz), 2.76 (dd, 1H, J = 8, 16 Hz), 3.48 (d, 2H, J = 14 Hz), 3.55 (m, 1H), 3.73 (d, 2H, J = 14 Hz), 6.56 (broad d, 2H J = 8 Hz), 6.70 (broad s, 2H), 6.77 (d, 2H, J = 7.5 Hz), 7.08 (t, 2H, J = 8 Hz); MS (ES+) m/z 478 (MNa+), 456 (base, MH+), 400, 344.

Example 11

N,N-bis(3-(4-Methylbenzoyl)aminobenzyl)Asp(O-t-Bu)-O-t-Bu

To a solution of 109 mg (0.24 mmol) of N,N-bis(3-aminobenzyl)Asp(O-t-Bu)-O-t-Bu, 29 mg (0.24 mmol) of DMAP, 125 μL (93 mg, 0.72 mmol) of DIEA in 1 mL of CH_2Cl_2 was added 95 μL (111 mg, 0.72 mmol) of 4-methylbenzoyl chloride. The solution was stirred under N_2 overnight and was then partitioned between EtOAc and water. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give 177 mg of yellow oil. The crude material was purified by MPLC using a solvent gradient ranging from 20–25% EtOAc/hexanes to provide 87 mg of N,N-bis(3-(4-methylbenzoyl)aminobenzyl)Asp(O-t-Bu)-O-t-Bu as a pale yellow oil; ^1H NMR ($CDCl_3$, 300 MHz) 1.36 (s, 9H), 1.55 (s, 9H), 2.35 (s, 6H), 2.53 (dd, 1H, J = 6, 16 Hz), 2.76 (dd, 1H, J = 9, 16 Hz), 3.69 (d, 2H, J = 14), 3.77 (dd, 1H, J = 6, 9 Hz), 3.83 (d, 2H, J = 14), 7.01 (m, 6H), 7.26 (t, 2H, J = 8 Hz), 7.59 (m, 6H), 8.11 (s, 2H), 8.49 (s, 2H); MS (ES+) m/z 714 (MNa+), 692 (base, MH+), 636, 580.

Example 12

N,N-bis(3-(4-Methylbenzoyl)aminobenzyl)Asp-OH (cpd 64)

A solution of 87 mg (0.13 mmol) of N,N-bis(3-(4-methylbenzoyl)aminobenzyl)Asp(O-t-Bu)-O-t-Bu in 1 mL of 50% TFA/CH_2Cl_2 was stirred overnight. The mixture was concentrated and the residue was dissolved in HOAc and freeze-dried to afford 77 mg **cpd 64** as a white solid; ^1H NMR (CD_3OD, 300 MHz) 2.40 (s, 6H), 2.85 (dd, 1H, J = 6, 16.5 Hz), 2.98 (dd, 1H, J = 8, 16.5 Hz), 4.02 (d, 2H, J = 13.5 Hz), 4.08 (d, 4H, J = 13.5 Hz), 4.10 (t, 1H, J = 6.5 Hz), 7.22 (m, 6H), 7.34 (t, 2H, J = 7.5 Hz), 7.60 (broad d, 2H, J = 9 Hz), 7.76 (d, 4H, J = 8 Hz), 7.88 (broad s, 2H); MS (ES+) m/z 580 (base, MH+).

Example 13

[N-Cbz-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2$]$_2$

To a solution of 1.69 g (5.0 mmol) of N-Cbz-Glu(O-t-Bu)-OH, 0.365 mL (0.371 g, 2.5 mmol) of 1,8-diamino-3,6-dioxaoctane, 0.743 g (5.5 mmol) of HOBT, and 1.05 mL (0.776 g, 6.0 mmol) of DIEA in 15 mL of CH_2Cl_2 was added 1.05 g (5.5 mmol) of EDCI in one portion. After stirring at room temperature under N_2 overnight, the mixture was partitioned between EtOAc and 10% aqueous citric acid. The organic layer was washed with water, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated to give 1.87 g of (N-Cbz-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2$)$_2$ as a colorless oil; ^{1}H NMR (CD_3OD, 300 MHz) 1.43 (s, 18H), 1.85 (m, 2H), 2.05 (m, 2H), 2.31 (t, 4H, J = 8 Hz), 3.37 (t, 4H, J = 5 Hz), 3.52 (t, 4H, J = 5 Hz), 3.58 (s, 4H), 4.15 (m, 2H), 5.09 (dd, 4H, J = 12, 16 Hz), 7.30 (m, 10H); MS (ES+) m/z 809 (base, MNa+), 787 (MH+).

Example 14

[Glu(O-t-Bu)-$NHCH_2CH_2OCH_2$]$_2$

Ammonium formate (0.78 g, 12.4 mmol) and 0.16 g of 10% palladium on carbon were added to a solution of (N-Cbz-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2$)$_2$ in 12 mL of MeOH and the resulting suspension was stirred under N_2 at room temperature overnight. The mixture was diluted with CH_2Cl_2 and filtered through a Celite pad. The solids were washed thoroughly with CH_2Cl_2 and the combined organic filtrates were concentrated to dryness. The residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$, washed with brine, dried over Na_2SO_4, and concentrated to give 1.13 g of (Glu(O-t-Bu)-$NHCH_2CH_2OCH_2$)$_2$ as a colorless oil; 1.44 (s, 18H), 1.81 (m, 2H), 2.08 (m, 2H), 2.35 (m, 4H), 3.39 (dd, 2H, J = 5, 7.5 Hz), 3.47 (t, 4H, J = 5 Hz), 3.58 (t, 4H, J = 5 Hz), 7.53 (m, 2H).

Example 15

[N,N-bis(4-Benzyloxybenzyl)Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ (cpd 245)

A solution of 199 mg (0.384 mmol) of [Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$, 403 mg (1.73 mmol) of 4-benzyloxybenzyl chloride, 30 mg (0.2 mmol) of NaI, and 334 L (248 mg, 1.92 mmol) of DIEA was stirred under N_2 at room temperature for several days. The solution was partitioned between EtOAc and water and the organic layer was washed three times with water and once with brine. After drying over Na_2SO_4, the solution was concentrated to give 528 mg of yellow oil which was purified by MPLC using a solvent gradient ranging from 42–50% EtOAc/hexanes to afford 318 mg (64%) of **cpd 245** as a white foam; ^1H NMR ($CDCl_3$, 300 MHz) 1.42 (s, 18H), 2.01 (m, 4H), 2.38 (m, 2H), 2.55 (m, 2H), 3.03 (dd, 2H, J = 5, 8 Hz), 3.31 (m, 2H), 3.4–3.6 (complex, 18H), 4.99 (s, 8H), 6.89 (d, 8H, J = 8.5), 7.1–7.4 (complex, 30H).

Example 16

[N,N-bis(4-Benzyloxybenzyl)GluNHCH$_2$CH$_2$OCH$_2$]$_2$ (cpd 246)

A solution of 219 mg (0.168 mmol) of **cpd 245** in 2 mL of 33% TFA/CH_2Cl_2 was stirred ad room temperature overnight. The mixture was concentrated to give a crude product which was dissolved in HOAc and freeze-dried to afford 251 mg of **cpd 246** as an amber oil; ^1H NMR (CD_3OD, 300 MHz) 2.1–2.6 (complex, 8H), 3.3–3.6 (complex, 8H), 3.57 (s, 4H), 3.78 (m, 2H), 4.25 (broad d, 4H, J = 14 Hz), 4.36 (broad d, 4H, J = 14 Hz), 5.09 (s, 8H), 7.03 (d, 8H, J = 8 Hz), 7.3–7.5 (complex, 28H); MS (ES+) m/z 1192 (MH+), 995, 596, 197 (base).

Example 17

[N-(3-Phenoxybenzyl)Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$

A solution of 680 mg (0.76 mmol) of [Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ and 278 μL (317 mg, 1.6 mmol) of 3-phenoxybenzaldehyde in 3 mL of TMOF was

stirred overnight at room temperature under N_2. The mixture was concentrated and pumped at high vacuum to give a colorless oil which was dissolved in 3 mL of CH_2Cl_2 and treated with 678 mg (3.2 mmol) of $NaBH(OAc)_3$. After stirring under N_2 for 2 days, 50 mL of saturated aqueous $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, and concentrated and the crude product (1.01 g) was purified by MPLC using a solvent gradient ranging from 2–4% MeOH/CH_2Cl_2 to afford 490 mg of [N-(3-phenoxybenzyl)Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ as a colorless oil; ^1H NMR ($CDCl_3$, 300 MHz) 1.41 (s, 18H), 1.89 (m, 4H), 2.31 (m, 4H), 3.12 (t, 2H, J = 6 Hz), 3.45 (m, 8H), 3.55 (s, 4H), 3.60 (d, 2H, J = 13.5 Hz), 3.73 (d, 2H, J = 13.5 Hz), 6.86 (dd, 2H, J = 1.5, 8 Hz), 7.00 (m, 8H), 7.2–7.4 (complex, 8H); MS (ES+) m/z 883 (MH+), 589, 442, 414, 386 (base), 183.

Example 18

[N-(3-Nitrobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$

DIEA (269 μL, 199 mg, 1.54 mmol), 3-nitrobenzyl bromide (322 mg, 1.49 mmol), and [N-(3-phenoxybenzyl)Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ (482 mg, 0.546 mmol) were combined in 2 mL of DMF and heated at 60–70 °C under N_2 for 2 days. The reaction mixture was cooled and partitioned between 100 mL of EtOAc and water. The organic layer was washed with three times with water and once with brine, dried over Na_2SO_4, and concentrated to give 661 mg (~100%) of [N-(3-nitrobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ which was used without purification; MS (ES+) m/z 1154 (MH+), 577, 130 (base).

Example 19

[N-(3-Aminobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$

A solution of 661 mg (0.54 mmol) of crude [N-(3-nitrobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ and 2.71 g (12.0 mmol) of $SnCl_2 \bullet 2\ H_2O$

in 20 mL of absolute EtOH was refluxed under N_2 for 30 min. The cooled solution was poured into 500 mL of 2.5% aqueous Na_2CO_3 with rapid stirring and the resulting cloudy mixture was extracted thoroughly with EtOAc. The slightly cloudy organic extracts were washed twice with brine, dried over Na_2SO_4, anc concentrated to give 604 mg of yellow oil which was purified by MPLC using 3% MeOH/CH_2Cl_2 to provide 350 mg (59%) of [N-(3-aminobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$ as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.41 (s, 18H), 1.97 (m, 4H), 2.25 (m, 4H), 2.48 (m, 4H), 3.03 (dd, 2H, J = 5, 8 Hz), 3.30 (m, 2H), 3.4–3.8 (complex, 24H), 6.47 (d, 2H, J = 7.5 Hz), 6.65 (m, 4H), 6.85 (d, 2H, J = 9.5 Hz), 6.9–7.15 (complex, 12H), 7.2–7.4 (complex, 8H); MS (ES+) m/z 1094 (MH+), 547 (base).

Example 20

[N-(3-Phenoxybenzyl)-N-(3-(pentanoylamino)benzyl)-Glu-$NHCH_2CH_2OCH_2]_2$ (cpd 247)

Pentanoyl chloride (16 uL, 16 mg, 0.136 mmol) was added dropwise to a solution of 68 mg (0.062 mmol) of [N-(3-aminobenzyl)-N-(3-phenoxybenzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$, 20 µL (20 mg, 0.25 mmol) of pyridine in 0.3 mL of 1,2-dichloroethane. The mixture was shaken under N_2 overnight and was then partitioned between EtOAc and water. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated to give 77 mg of [N-(3-phenoxybenzyl)-N-(3-(pentanoylamino)benzyl)-Glu(O-t-Bu)-$NHCH_2CH_2OCH_2]_2$; MS (ES+) m/z 1073, 575 (base, MH+/2). The crude product was dissolved in 1 mL of 50% TFA/CH_2Cl_2 and allows to stand overnight. The solution was concentrated and the resulting oil was dissolved in HOAc and freeze-dried to provide 82 mg of **cpd 247**; ^{1}H NMR (CD_3OD, 300 MHz) 3.98 (t, 6H, J = 7.5 Hz), 1.39 (sextet, 4H, J = 7.5 Hz), 1.66 (quintet, 4H, J = 7.5 Hz), 1.65 (m, 2H), 1.78 (m, 2H), 2.35 (t, 4H, J = 7.5 Hz), 2.45 (m, 4H), 3.38 (m, 4H), 3.50 (t, 2H, J = 5), 3.57 (m, 4H), 4.10 (broad s,

8H), 6.9–7.25 (complex, 14H), 7.25–7.4 (complex, 10H), 7.71 (s, 2H); MS (ES+) m/z 1150 (MH+), 575 (base).

Example 21

$[N\text{-Cbz-Lys(Boc)-NHCH}_2\text{CH}_2]_3\text{N}$

A solution of 1.0 g (2.63 mmol) of N-Cbz-Lys(Boc)OH, 0.131 mL (0.128 g, 0.876 mmol) of tris(2-aminoethyl)amine, 0.391 g (2.98 mmol) of HOBt, 0.555 g (2.89 mmol) of EDCI, and 0.55 mL (0.408 g, 3.16 mmol) of DIEA in 5 mL of CH_2Cl_2 was stirred under N_2 at room temperature overnight. The mixture was diluted with EtOAc and washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine. The solution was dried over Na_2SO_4 and concentrated to give 0.872 g of $[N\text{-Cbz-Lys(Boc)-NHCH}_2\text{CH}_2]_3\text{N}$ as an off-white solid; ^{1}H NMR (CD_3OD, 300 MHz) 135 (m, 12H), 1.40 (s, 27H), 1.60 (m, 3H), 1.72 (m, 3H), 2.51 (m, 6H), 2.99 (m, 6H), 3.10 (m, 3H), 3.21 (m, 3H), 4.12 (m, 3H), 5.00 (d, 3H, J = 12.5 Hz), 5.08 (d, 3H, J = 12.5 Hz), 7.29 (m, 15H); MS (ES+) m/z 1243 (base, MH+), 567, 467.

Example 22

$[\text{Lys(Boc)-NHCH}_2\text{CH}_2]_3\text{N}$

A solution of 0.841 g (0.682 mmol) $[N\text{-Cbz-Lys(Boc)-NHCH}_2\text{CH}_2]_3\text{N}$, 0.252 g of 10% Pd-C, and 0.774 g (12.3 mmol) of ammonium formate in 10 mL of MeOH was stirred for 5 h at room temperature under N_2. The mixture was filtered through a Celite pad, the solids were washed with CH_2Cl_2, and the reslulting solution was concentrated to dryness. The residue was partitioned between CH_2Cl_2 and brine; the organic layer was dried over Na_2SO_4 and concentrated to provide 0.191 g of $[\text{Lys(Boc)-NHCH}_2\text{CH}_2]_3\text{N}$ as an off-white solid; ^{1}H NMR (CD_3OD, 300 MHz) 1.40 (s, 27H), 1.45 (m, 12H), 1.75 (m, 6H), 2.62 (m, 6H), 3.01 (m, 6H), 3.28 (m, 6H), 3.64 (m, 3H); MS (ES+) m/z 853 (MNa+), 831 (MH+), 266 (base).

Example 23

[N,N-bis(3-Phenoxybenzyl)Lys(Boc)-$NHCH_2CH_2]_3N$

A solution of 65 mg (0.078 mmol) of [Lys(Boc)-$NHCH_2CH_2]_3N$, 120 μL (140 mg, 0.70 mmol) of 3-phenoxybenzaldehyde, and 71 μL (65 mg, 0.70 mmol) of borane-pyridine complexin 3 mL of absolute EtOH was stirred for 4 days at room temperature under N_2. The mixture was concentrated to dryness and partitioned between water and CH_2Cl_2. The organic layer was concentrated to give a yellow oil which was purified by MPLC using 2.5% MeOH/CH_2Cl_2 to give 78 mg of [N,N-bis(3-phenoxybenzyl)Lys(Boc)-$NHCH_2CH_2]_3N$ as a yellow oil; MS (ES+) m/z 872 (base, [M-$C_{13}H_{12}O$)/2]+), 611, 443.

Example 24

[N,N-bis(3-Phenoxybenzyl)Lys-$NHCH_2CH_2]_3N$ (cpd 277)

A solution of 78 mg (0.048 mmol) of [N,N-bis(3-phenoxybenzyl)Lys(Boc)-$NHCH_2CH_2]_3N$ in 2 mL of 50% TFA/CH_2Cl_2 was stirred for 2 h at room temperature. The mixture was diluted with CH_2Cl_2, washed with water and 5% Na_2CO_3, and concentrated to give 57 mg of **cpd 277** as an off-white foam; ^1H NMR (CD_3OD, 300 MHz) 1.35 (m, 6H), 1.52 (m, 6H), 1.76 (m, 6H), 2.75 (m, 6H), 3.19 (m, 6H), 3.40 (m, 6H), 3.60 (m, 9H), 3.77 (m, 6H), 6.79 (d, 6H, J = 8 Hz), 6.93 (m, 24H), 7.05 (m, 6H), 7.19 (m, 6H), 7.29 (m, 12H); MS (ES+) m/z 813 ([MH_2/2]+), 721, 542 (base, [MH/3]+).

Example 25

N,N-bis(3-Phenoxycinnamyl)Ser(t-Bu)-OMe (cpd 290) and N-(3-phenoxycinnamyl)Ser(t-Bu)-OMe (cpd 352)

A solution of 423 mg (2.0 mmol) of H-Ser(t-Bu)OMe•HCl, 1.01 g (3.5 mmol) of 3-phenoxycinnamyl bromide (Jackson, W. P.; Islip, P. J.; Kneen, G.;

Pugh, A.; Wates, P. J. *J.Med.Chem.* **31** *1988*; 499-500), and 0.87 mL (5.0 mmol, 650 mg) of DIEA in 6 mL of DMF was stirred under N_2 at room temperature for 20 h. The mixture was partitioned between EtOAc and water and the organic layer was washed with water and brine. After drying over Na_2SO_4, the organic solution was concentrated to give 0.98 g of yellow oil. The crude residue was purified by MPLC using a solvent gradient ranging from 10–30% EtOAc/hexanes to give two products. The less polar product (168 mg, 14% based on starting amino acid), N,N-bis(3-phenoxycinnamyl)Ser(t-Bu)-OMe (**cpd 290**), was isolated as a pale yellow oil; ^1H NMR ($CDCl_3$, 300 MHz) 1.15 (s, 9H), 3.35 (dd, 2H, J = 7, 14.5 Hz), 3.53 (dd, 2H, J = 5.5, 14.5 Hz), 3.6–3.8 (complex, 3H), 3.69 (s, 3H), 6.18 (dt, 2H, J = 16, 6.5 Hz), 6.49 (d, 2H, J = 16 Hz), 6.86 (dd, 2H, J = 2, 8 Hz), 6.9–7.4 (complex, 16H); MS (ES+) m/z 614, 592 (MH+, base), 406, 384, 209.

The more polar product (354 mg, 46% based on starting amino acid), N-(3-phenoxycinnamyl)Ser(t-Bu)-OMe (**cpd 352**), was isolated as a pale yellow oil; ^1H NMR ($CDCl_3$, 300 MHz) 1.15 (s, 9H), 1.98 (broad s, 1H), 3.32 (ddd, 1H, J = 1.2, 6.5, 14 Hz), 3.4–3.7 (complex, 4H), 3.72 (s, 3H), 6.21 (dt, 1H, J = 16, 6.5 Hz), 6.48 (d, 1H, J = 16 Hz), 6.88 (dd, 1H, J = 1.5, 8 Hz), 7.0–7.4 (complex, 8H); MS (ES+) m/z 789 (2M+Na+), 384 (MH+, base), 209.

Example 26

N,N-Bis(3-phenoxycinnamyl)Ser-OMe (cpd 299)

N,N-Bis(3-phenoxycinnamyl)Ser(t-Bu)-OMe (**cpd 290**, 168 mg, 0.284 mmol) was stirred in 3 mL of 50% TFA/CH_2Cl_2 under N_2 overnight. The solvent was removed using a rotary evaporator and the crude residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$. After washing with brine and drying over Na_2SO_4, the organic layer was concentrated using a rotary evaporator and the crude product (134 mg) was purified by MPLC using 30% EtOAc/hexanes to give 44 mg (29%) of N,N-bis(3-phenoxycinnamyl)Ser-OMe (**cpd 299**) as a

colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.6 (broad s, 2H), 3.38 (dd, 2H, J - 8, 12 Hz), 3.4–3.9 (complex, 5H), 3.72 (s, 3H), 6.13 (dt, 2H, J = 16, 7 Hz), 6.50 (d, 2H, J = 16 Hz), 6.8–7.4 (complex, 18H); MS (ES+) m/z 536 (MH+).

Example 27

N,N-Bis(3-phenoxycinnamyl)Ser-OH (cpd 300)

N,N-Bis(3-phenoxycinnamyl)Ser-OMe (**cpd 299**, 44 mg, 0.082 mmol) was dissolved in 0.2 mL of MeOH and was stirred with 0.090 mL of 1N aqueous NaOH. When TLC analysis revealed that starting material had been consumed, the solvent was removed by rotary evaporation and the residue was lyophilized from acetic acid to give 42 mg (88%) of N,N-bis(3-phenoxycinnamyl)Ser-OH acetate (**cpd 300**) as a sticky yellow solid; ^{1}H NMR (methanol-d_4, 300 MHz) 1.97 (s, 3H), 3.3–4.2 (complex, 7H), 6.80 (d, 2H, J = 16 Hz), 6.9–7.4 (complex, 18H); MS (ES+) m/z 522 (MH+), 209.

Example 28

N-(3-Phenoxycinnamyl)Ser-OMe (cpd 346)

N-(3-Phenoxycinnamyl)Ser(t-Bu)-OMe (**cpd 352**, 268 mg, 0.699 mmol) was stirred in 3 mL of 50% TFA/CH_2Cl_2 under N_2 overnight. The solvent was removed using a rotary evaporator and the crude residue (256 mg) was purified by MPLC using EtOAc to give 137 mg (60%) of N-(3-phenoxycinnamyl)Ser-OMe (**cpd 346**) as a colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 2.2 (broad s, 2H), 3.36 (dd, 1H, J = 6, 14 Hz), 3.4–3.5 (complex, 2H), 3.62 (dd, 1H, J = 6.5, 11 Hz), 3.74 (s, 3H), 3.80 (dd, 1H, J = 4.5, 11 Hz). 6.19 (dt, 1H, J = 16, 6.5 Hz), 6.48 (d, 1H, J = 6 Hz), 6.88 (dd, 1H, J = 1.5, 8 Hz), 7.0–7.4 (complex, 8H); MS (ES+) m/z 677 (2M+Na+), 350 (M+Na+), 328 (MH+), 209 (base).

Example 29

N-(3-phenoxycinnamyl)Ser-OH (cpd 347)

N-(3-Phenoxycinnamyl)Ser-OMe (**cpd 346**, 110 mg, 0.336 mmol) was dissolved in 1.5 mL of MeOH and was stirred with 0.50 mL of 1N aqueous NaOH. When TLC analysis revealed that starting material had been consumed, the solvent was removed by rotary evaporation. The residue was dissolved in water and acidified to pH 7–8 with 1N aqueous HCl; the resulting solids were filtered, washed with water, and dried to give 71 mg of white powder. The insoluble powder was dissolved in TFA and, after removal of excess TFA by rotary evaporation, lyophilized from acetic acid to give 82 mg (57%) of N-(3-phenoxycinnamyl)Ser-OH trifluoroacetate (**cpd 347**) as an amber oil; ^{1}H NMR (methanol-d_4, 300 MHz) 3.88 (d, 2H, J = 7H), 4.0–4.2 (complex, 3H), 6.27 (dt, 1H, J = 16, 6.5), 6.83 (d, 1H, J = 16 Hz), 6.9–7.4 (complex, 9H); MS (ES+) m/z 314, (MH+), 209.

Example 30

N-(3-Phenoxycinnamyl)Glu(O-t-Bu)-OH (cpd 337)

A mixture of 249 mg (0.585 mmol) of N-(3-phenoxycinnamyl)Glu(O-t-Bu)-OMe (**cpd 334**) in 3 mL of MeOH was sonicated to speed dissolution, and the resulting solution was treated with 0.585 mL of 1N aqueous NaOH. After stirring overnight, the MeOH was removed using a rotary evaporator and the residue was dissolved in water. Acidification with 0.64 mL of 1N aqueous HCl produced a 250 mg of solid material that was triturated with Et_2O to give 111 mg (46%) of N-(3-phenoxycinnamyl)Glu(O-t-Bu)-OH (**cpd 337**) as a white solid; ^{1}H NMR (300 MHz, methanol-d_4) 1.43 (s, 9H), 1.9–2.2 (complex, 2H), 2.46 (t, 2H, J = 7 Hz), 3.57 (dd, 1H, J = 5, 7 Hz), 3.78 (dd, 1H, J = 7, 13.5 Hz), 3.82 (dd, 1H, J = 7, 13.5 Hz), 6.28 (dt, 1H, J = 16, 7 Hz), 6.81 (d, 1H, J = 16 Hz), 6.9–7.5 (complex, 9H); MS (ES+) m/z 412 (MH+, base), 356, 209. Anal. Calcd for $C_{24}H_{29}NO_5 \cdot 0.4\ H_2O$: C, 68.55; H, 7.04; N, 3.24. Found: C, 68.89; H, 7.04; N, 3.24.

Example 31

N-(3-Phenoxycinnamyl)Glu-OH (cpd 326)

A mixture of 85 mg (0.21 mmol) of N-(3-phenoxycinnamyl)Glu(O-t-Bu)-OH (**cpd 337**) in was stirred in 1 mL of 50% TFA/CH_2Cl_2 for 1 h. After solvent removal using a rotary evaporator, the residue was dissolved in acetic acid and freeze-dried to give 75 mg (76%) of N-(3-phenoxycinnamyl)Glu-OH tifluoroacetate (**cpd 326**) as a fluffy white solid; ^{1}H NMR (300 MHz, methanol-d_4) 2.0–2.4 (complex, 2H), 2.55 (m, 2H), 3.84 (d, 2H, J = 7 Hz), 3.96 (dd, 1H, J = 5, 7 Hz, 6.24 (dt, 1H, J = 16, 7 Hz), 6.84 (d, 1H, J = 16 Hz), 6.9–7.4 (complex, 9H); MS (ES+) m/z 356 (MH+), 209 (base).

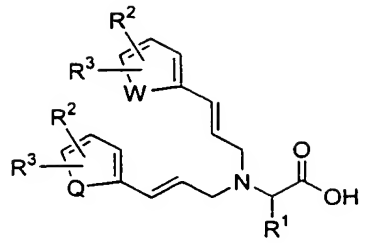


Table 2

cpd	% inh	R^1 (amino acid side chain)	R^2	R^3	W, Q
11	70	Asn, Asp, Gln, Glu	3-PhO		CH=CH
12	59	Cys, Met, Ser, Thr	3-PhO		CH=CH
13	nd	Arg, Gly, His, Pro	3-PhO		CH=CH
14	30	Lys(2-Cl-Cbz), Phe, Trp, Tyr	3-PhO		CH=CH
15	48	Ala, Ile, Leu, Val	3-PhO		CH=CH
16	nd	Glu, Asp	2,3-benzo		CH=CH
17	nd	Cys, Met	2,3-benzo		CH=CH
18	nd	Ser, Thr	2,3-benzo		CH=CH
19	nd	His, Arg(Mtr)	2,3-benzo		CH=CH
20	nd	Pro, Gly	2,3-benzo		CH=CH
21	nd	Phe, Tyr	2,3-benzo		CH=CH
22	nd	Trp, Lys(2-Cl-Cbz)	2,3-benzo		CH=CH
23	nd	Ile, Ala	2,3-benzo		CH=CH
24	nd	Val,Leu	2,3-benzo		CH=CH
25	nd	Asn, Lys	2,3-benzo		CH=CH
26	nd	Ala, Ile		3,4-benzo	CH=CH
27	nd	Arg(Mtr), Lys(2-Cl-Cbz)		3,4-benzo	CH=CH
28	nd	Asp, Glu		3,4-benzo	CH=CH
29	nd	Cys, Met		3,4-benzo	CH=CH
30	nd	Gly, Pro		3,4-benzo	CH=CH
31	nd	His, Lys		3,4-benzo	CH=CH
32	nd	Leu, Val		3,4-benzo	CH=CH

cpd	% inh	R^1 (amino acid side chain)	R^2	R^3	W, Q
33	nd	Lys(2-Cl-Cbz), Phe		3,4-benzo	CH=CH
34	nd	Ser, Thr		3,4-benzo	CH=CH
35	nd	Trp, Tyr		3,4-benzo	CH=CH

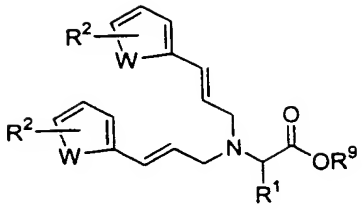


Table 3

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^9	W, Q	MS MH+
36	nd	CH_3	4-CF_3	H	CH=CH	458
37	19	H	4-CF_3	H	CH=CH	430
38	nd	$(CH_2)_4NH$(2-Cl-Cbz)	4-F	H	CH=CH	448
40	nd	CH_3	4-F	H	CH=CH	223
41	nd	CH_2CO_2H	4-F	H	CH=CH	266
42	nd	$CH_2CH_2CO_2H$	4-F	H	CH=CH	281
43	nd	$(CH_2)_3NHC(=NH)NH_2$	4-F	H	CH=CH	308
45	nd	$PhCH_2$	4-F	H	CH=CH	299
46	nd	4-HO-$PhCH_2$	4-F	H	CH=CH	315
47	nd	CH_2OH	4-F	H	CH=CH	238
48	nd	$CH(OH)CH_3$	4-F	H	CH=CH	253
49	1	$(CH_2)_3NHC(=NH)NH_2$	H	H	S	419
50	-6	$(CH_2)_4NH_2$	H	H	S	391
51	nd	$CH(CH_3)CH_2CH_3$	H	H	S	376
52	21	$CH_2CH_2CO_2H$	H	H	S	392
53	14	CH_2CO_2H	H	H	S	378
54	18	CH_3	H	H	S	334
55	4	$CH_2CH_2CONH_2$	H	H	S	391
56	nd	$(CH_2)_4NHCbz$	H	Me	S	539
57	0	$(CH_2)_4NHCbz$	H	CH_2Ph	S	615
58	nd	CH_2(indol-3-yl)	H	Me	S	463
59	26	$CH_2CH_2CO_2$-t-Bu	H	Me	S	462
60	9	$CH_2CH_2CO_2Et$	H	Me	S	434

61	14	$CH_2CH_2CO_2H$	H	Me	S	406

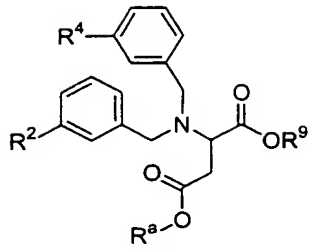


Table 4

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^4	R^9	MS, MH+
62	nd	t-Bu	NO_2	NO_2	t-Bu	516
63	20	H	$PhCH_2NH$	PhO	H	511
64	-4	H	4-MePhCONH	4-MePhCONH	H	580
65	-7	H	$4\text{-MePhSO}_2\text{NH}$	$4\text{-MePhSO}_2\text{NH}$	H	652
66	-16	H	$3\text{-ClPhCH}_2\text{NH}$	PhO	H	546
67	-8	H	$3\text{-BrPhCH}_2\text{NH}$	PhO	H	590
68	-13	H	$2\text{-FPhCH}_2\text{NH}$	PhO	H	529
69	-13	H	$2\text{-MePhCH}_2\text{NH}$	PhO	H	525
70	-8	H	$4\text{-FPhCH}_2\text{NH}$	PhO	H	529
71	-6	H	$3\text{-ClPhCH}_2\text{NH}$	4-Me-PhO	H	560
72	-14	H	$F_5\text{-PhCH}_2\text{NH}$	4-Me-PhO	H	615
73	-13	H	$2\text{-FPhCH}_2\text{NH}$	4-Me-PhO	H	543
74	-7	H	$3\text{-CNPhCH}_2\text{NH}$	4-Me-PhO	H	550
75	-2	H	$PhCH_2NH$	4-Me-PhO	H	525

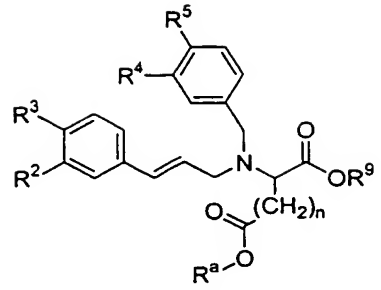


Table 5

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	R^9	n	MS, MH+
76	25	t-Bu	PhO	H	PhO	H	t-Bu	1	636
77	52	H	PhO	H	PhO	H	H	1	524
78	nd	H	H	4-MePhCONH	H	BnO	H	2	593
79	nd	H	H	n-BuCONH	H	BnO	H	2	559
80	nd	H	H	2-naphthyl CONH	H	BnO	H	2	629
81	nd	H	H	2-furyl CONH	H	BnO	H	2	569
82	32	H	H	4-MeO-PhCONH	H	BnO	H	2	609
83	18	H	H	$HO_2C(CH_2)_3CONH$	H	BnO	H	2	589
84	14	H	H	C_2F_5CONH	H	BnO	H	2	621
85	20	H	H	CF_3CONH	H	BnO	H	2	571
86	37	H	H	4-pyridyl-CONH	H	BnO	H	2	580
87	23	H	H	$4\text{-}MePhSO_2NH$	H	BnO	H	2	629
88	10.3	H	H	HO_2CCH_2(1,1-cyclopentyl) CH_2CONH	H	BnO	H	2	643
89	22	H	H	PhOCONH	H	BnO	H	2	595
90	29	H	H	4-Ph-PhCONH	H	BnO	H	2	655
91	19	H	H	$4\text{-}NO_2\text{-}PhCONH$	H	BnO	H	2	624

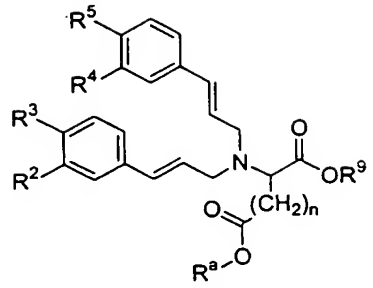


Table 6

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	R^6	R^9	MS, MH+
92	20	H	H	H	H	H	2	Me	394
93	20	t-Bu	H	H	H	H	2	Me	450
94	25	Et	H	H	H	H	2	Me	422
95	15	t-Bu	2,3-benzo		2,3-benzo		2	Me	550
96	-5	t-Bu	PhO	H	PhO	H	2	Me	634
97	14	t-Bu	3,4-benzo		3,4-benzo		2	H	536
98	12	t-Bu	H	Ph	H	Ph	2	Me	602
99	13	t-Bu	3,4-di-Cl-PhO	H	3,4-di-Cl-PhO	H	2	Me	772
100	34	H	H	Ph	H	Ph	2	Me	546
101	32	H	3,4-di-Cl-PhO	H	3,4-di-Cl-PhO	H	2	Me	716
102	5	t-Bu	4-t-Bu-PhO	H	4-t-Bu-PhO	H	2	t-Bu	789
103	17	t-Bu	3-CF3-PhO	H	3-CF3-PhO	H	2	t-Bu	812
104	78	H	4-t-Bu-PhO	H	4-t-Bu-PhO	H	2	H	676
105	70	H	3-CF3-PhO	H	3-CF3-PhO	H	2	H	700
106	20	t-Bu	PhO	H	PhO	H	1	t-Bu	662
107	78	H	PhO	H	PhO	H	2	H	562*
108	81	H	PhO	H	PhO	H	1	H	550

*$[M-H]^-$

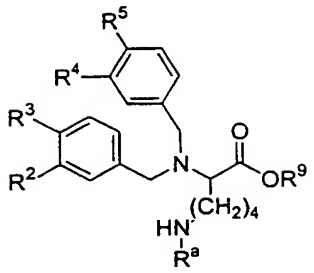


Table 7

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	R^9	MS, MH +
109	7	Boc	BnO	H	BnO	H	Me	653
110	54	H	H	BnO	H	BnO	Me	553
111	5	Boc	H	BnO	H	BnO	Me	653
112	59	H	BnO	H	BnO	H	Me	553
113	24	Boc	H	BnO	NO_2	H	Me	592
114	37	H	H	BnO	NO_2	H	Me	492
115	35	H	H	BnO	NH_2	H	Me	462
116	32	H	H	BnO	n-BuCONH	H	Me	546
117	34	H	H	BnO	2-furylCONH	H	Me	556
118	36	H	H	BnO	4-MePhCONH	H	Me	580
119	34	H	H	BnO	i-Pr-CONH	H	Me	532
120	35	H	H	BnO	4-pyridyl-CONH	H	Me	567
121	45	H	H	BnO	2-naphthyl-CONH	H	Me	616
122	nd	Boc	$PhCH_2NH$	H	$PhCH_2NH$	H	Me	651
123	nd	Boc	2-$MePhCH_2NH$	H	2-$MePhCH_2NH$	H	Me	679
124	nd	Boc	4-MeO-$PhCH_2NH$	H	4-MeO-$PhCH_2NH$	H	Me	711
125	nd	Boc	3,4-di-MeO-$PhCH_2NH$	H	3,4-di-MeO-$PhCH_2NH$	H	Me	771
126	nd	Boc	$-NH_2$	H	$-NH_2$	H	Me	471

127	nd	H	$PhCH_2NH$	H	$PhCH_2NH$	H	Me	551
128	nd	H	2-$MePhCH_2NH$	H	2-$MePhCH_2NH$	H	Me	579
129	nd	H	4-MeO-$PhCH_2NH$	H	4-MeO-$PhCH_2NH$	H	Me	611
130	nd	H	3,4-di-MeO-$PhCH_2NH$	H	3,4-di-MeO-$PhCH_2NH$	H	Me	671
131	nd	H	$PhCH_2CH_2NH$	H	$PhCH_2CH_2NH$	H	Me	579
132	nd	HO_2CCH_2 CH_2CO	$PhCH_2NH$	H	$PhCH_2NH$	H	Me	651
133	nd	HO_2CCH_2 CH_2CO	2-$MePhCH_2NH$	H	2-$MePhCH_2NH$	H	Me	679
134	nd	HO_2CCH_2 CH_2CO	4-MeO-$PhCH_2NH$	H	4-MeO-$PhCH_2NH$	H	Me	711
135	nd	HO_2CCH_2 CH_2CO	3,4-di-MeO-$PhCH_2NH$	H	3,4-di-MeO-$PhCH_2NH$	H	Me	771
136	nd	HO_2CCH_2 CH_2CO	$PhCH_2CH_2NH$	H	$PhCH_2CH_2NH$	H	Me	679

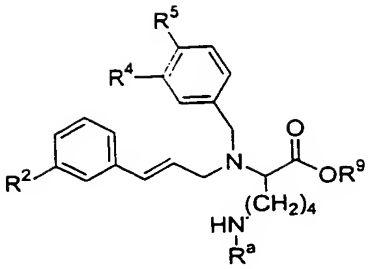


Table 8

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^4	R^5	R^9	MS, MH+
137	nd	H	PhO	PhO	H	Me	551
138	nd	Boc	4-t-Bu-PhO	BnO	H	Me	721
139	nd	H	4-t-Bu-PhO	BnO	H	Me	621
140	nd	H	$(CF_3CO)_2N$	BnO	H	H	666
141	nd	H	PhCONH	BnO	H	H	578
142	nd	H	4-pyridyl-CONH	BnO	H	H	579
143	nd	H	$(CF_3CO)_2N$	PhO	H	H	652
144	nd	H	PhCONH	PhO	H	H	564
145	nd	H	4-pyridyl-CONH	PhO	H	H	565
146	nd	H	$(CF_3CO)_2N$	MeO	MeO	H	620
147	nd	H	PhCONH	MeO	MeO	H	532
148	nd	H	4-pyridyl-CONH	MeO	MeO	H	533
149	nd	H	$(CF_3CO)_2N$	H	PhO	H	652
150	nd	H	PhCONH	H	PhO	H	564
151	nd	H	4-pyridyl-CONH	H	PhO	H	565
152	nd	H	PhCONH	H	BnO	H	578
153	nd	H	4-pyridyl-CONH	H	BnO	H	579
154	nd	H	$(CF_3CO)_2N$	H	BnO	H	666
155	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhCONH	PhO	H	H	694
156	nd	$HO_2CCH_2CH_2CO$	PhCONH	PhO	H	H	664
157	nd	$HO_2CCH_2CH_2CO$	2-naphthyl-CONH	PhO	H	H	714

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^4	R^5	R^9	MS, MH+
158	nd	$HO_2CCH_2CH_2CO$	4-Me-$PhSO_2NH$	PhO	H	H	714
159	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhCONH	2,3-benzo		H	652
160	nd	$HO_2CCH_2CH_2CO$	PhCONH	2,3-benzo		H	622
161	nd	$HO_2CCH_2CH_2CO$	2-naphthyl-CONH	2,3-benzo		H	672
162	nd	$HO_2CCH_2CH_2CO$	4-Me-$PhSO_2NH$	2,3-benzo		H	672
163	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhCONH	H	F	H	620
164	nd	$HO_2CCH_2CH_2CO$	PhCONH	H	F	H	590
165	nd	$HO_2CCH_2CH_2CO$	2-naphthyl-CONH	H	F	H	640
166	nd	$HO_2CCH_2CH_2CO$	4-Me-$PhSO_2NH$	H	F	H	640
167	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhCONH	BnO	H	H	708
168	nd	$HO_2CCH_2CH_2CO$	PhCONH	BnO	H	H	678
169	nd	$HO_2CCH_2CH_2CO$	2-naphthyl-CONH	BnO	H	H	728
170	nd	$HO_2CCH_2CH_2CO$	4-Me-$PhSO_2NH$	BnO	H	H	728

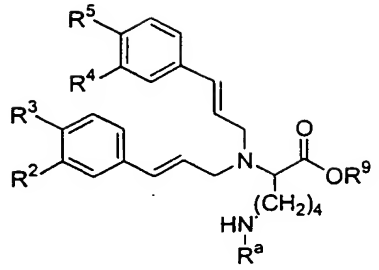


Table 9

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	R^9	MS, MH +
171	nb	Cbz	H	H	H	H	Me	527
172	15	Cbz	H	H	H	H	H	513
173	5	Cbz	H	H	H	H	t-Bu	569
174	23	Cbz	H	MeO	H	MeO	Me	587
175	1	Cbz		3,4-benzo		3,4-benzo	Me	627
176	-4	Cbz	PhO	H	PhO	H	Me	711
177	nd	Cbz	2,3-benzo		2,3-benzo		Me	627
178	36	Boc	H	NO_2	H	NO_2	Me	583
179	30	Boc	H	NO_2	H	NO_2	H	569
180	-4	Boc	PhO	H	PhO	H	Me	677
181	-9	Boc	4-t-Bu-PhO	H	4-t-Bu-PhO	H	Me	790
182	18	H	4-t-Bu-PhO	H	4-t-Bu-PhO	H	Me	689
183	36	Boc	NO_2	H	NO_2	H	Me	583
184	53	H	NO_2	H	NO_2	H	Me	483
185	29	H	NH_2	H	NH_2	H	Me	423
186	nd	H	n-Bu-CONH	H	n-Bu-CONH	H	Me	591
187	nd	H	2-furyl-CONH	H	2-furyl-CONH	H	Me	611
188	nd	H	PhCONH	H	PhCONH	H	Me	631

cpd	EPO / EBP-Ig %inh @ 50 µM	R^a	R^2	R^3	R^4	R^5	R^9	MS, MH +
189	nd	H	4-Me-PhCONH	H	4-Me-PhCONH	H	Me	659
190	nd	H	4-NO_2-PhCONH	H	4-NO_2-PhCONH	H	Me	721
191	nd	H	4-Me-PhSO_2NH	H	4-Me-PhSO_2NH	H	Me	731
192	nd	H	Cbz-NH	H	Cbz-NH	H	Me	691
193	nd	H	4-Br-PhCONH	H	4-Br-PhCO	H	Me	789
194	nd	H	2-MeO-PhCONH	H	2-MeO-PhCONH	H	Me	691
195	nd	H	3-MeO-PhCONH	H	3-MeO-PhCONH	H	Me	691
196	nd	H	4-MeO-PhCONH	H	4-MeO-PhCONH	H	Me	691
197	nd	H	CH_3CH=CHCONH	H	CH_3CH=CHCONH	H	Me	559
198	nd	H	C_2F_5CONH	H	C_2F_5CONH	H	Me	715
199	nd	H	2-naphthyl-CONH	H	2-naphthyl-CONH	H	Me	731
200	nd	H	$EtO_2CCH_2CH_2$CONH	H	$EtO_2CCH_2CH_2$CONH	H	Me	679
201	nd	H	CF_3CONH	H	CF_3CONH	H	Me	615
202	nd	H	$MeSO_2$NH	H	$MeSO_2$NH	H	Me	579

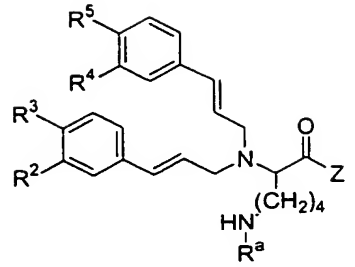


Table 10

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	Z	MS, MH +
203	37	Boc	H	H	H	H	4-(MeCOCH$_2$CH$_2$)-PhNH	640
204	-6	H	H	H	H	H	4-(MeCOCH$_2$CH$_2$)-PhNH	540
205	26	H	H	H	H	H	n-Bu-NH	434
206	17	2-MeO-PhCO	H	H	H	H	n-Bu-NH	568
207	20	4-MeO-PhCO	H	H	H	H	n-Bu-NH	568
208	22	PhCO	H	H	H	H	n-Bu-NH	538
209	25	2-MeO-PhCO	H	H	H	H	n-Bu-NH	568
210	nd	Boc	H	H	H	H	4-MeO-PhCH$_2$CH$_2$NH	612
211	62	H	H	H	H	H	4-MeO-PhCH$_2$CH$_2$NH	512
212	-10	H	H	H	H	H	n-Pr-NH	420
214	nd	Boc	H	H	H	H	3,4-di-MeO-PhCH$_2$CH$_2$NH	642
215	nd	Boc	H	H	H	H	3-MeO-PhCH$_2$CH$_2$NH	612
216	10	Boc	H	H	H	H	4-(PhCH=CHCH$_2$O)-PhCH$_2$NH	700
217	nd	Boc	H	H	H	H	4-HO-PhCH$_2$NH	584
218	nd	Boc	H	H	H	H	EtNH	506
219	nd	Boc	H	H	H	H	MeNH	492
220	45	H	H	H	H	H	4-(PhCH=CHCH$_2$O)-PhCH$_2$NH	600
221	48	H	H	H	H	H	3,4-di-MeO-PhCH$_2$CH$_2$NH	542

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	Z	MS, MH +
222	56	H	H	H	H	H	3-MeO-$PhCH_2CH_2NH$	512
223	nd	Boc	H	H	H	H	2-MeO-$PhCH_2CH_2NH$	612
224	51	H	H	H	H	H	2-MeO-$PhCH_2CH_2NH$	512
225	10	Boc	PhO	H	PhO	H	4-MeO-$PhCH_2CH_2NH$	797
226	nd	Boc	H	H	H	H	$PhCH_2CH_2NH$	582
227	48	H	H	H	H	H	$PhCH_2CH_2NH$	482
228	21	PhNHCO	PhO	H	PhO	H	4-MeO-$PhCH_2CH_2NH$	816
229	22	4-PhO-PhNHCO	H	H	H	H	4-MeO-$PhCH_2CH_2NH$	723
230	42	3,4-di-Cl-PhNHCO	H	H	H	H	4-MeO-$PhCH_2CH_2NH$	700
231	36	4-EtO2C-PhNHCO	H	H	H	H	4-MeO-$PhCH_2CH_2NH$	703
232	14	4-PhO-PhNHCO	PhO	H	PhO	H	4-MeO-$PhCH_2CH_2NH$	908
233	18	H	H	NO_2	H	NO_2	3-MeO-$PhCH_2CH_2NH$	602
234	nd	Boc	H	H	H	H	$PhCH_2NH$	568
235	49	H	H	H	H	H	$PhCH_2NH$	468
236	nd	Boc	H	Ph	H	Ph	4-MeO-$PhCH_2CH_2NH$	765
237	55	$HO_2CCH_2CH_2CO$	H	H	H	H	3-MeO-$PhCH_2CH_2NH$	612
238	39	H	H	Ph	H	Ph	4-MeO-$PhCH_2CH_2NH$	664
239	46	H	PhO	H	PhO	H	$PhCH_2CH_2NH$	666
240	nd	$HO_2CCH_2CH_2CH_2CO$	PhO	H	PhO	H	$PhCH_2CH_2NH$	780
285	40	H	H	H	H	H	4-(NH_2CO)piperidin-1-yl	489

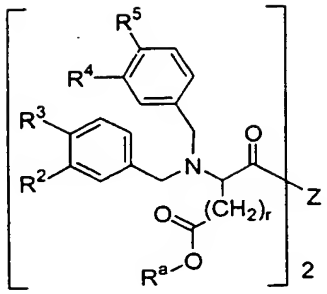


Table 11

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^3	R^4	R^5	Z	r	MS, $[MH_2/2]+$
241	2	t-Bu	H	BnO	H	BnO	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	1	666
242	1	t-Bu	H	BnO	H	BnO	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	1	674
243	75	H	H	BnO	H	BnO	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	1	610
244	66	H	H	BnO	H	BnO	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	1	618
245	0	t-Bu	H	BnO	H	BnO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	652
246	79	H	H	BnO	H	BnO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	596
247	47	H	n-Bu-CONH	H	PhO	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	575
248	56	H	2-furyl-CONH	H	PhO	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	585
249	72	H	4-Me-PhCONH	H	PhO	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	609
250	78	H	4-Me-$PhSO_2NH$	H	PhO	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	645

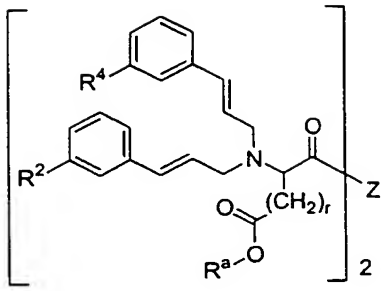


Table 12

cpd	EPO / EBP-Ig %inh @ 50 µM	R^a	R^2	R^4	Z	r	MS, $[MH_2/2]+$
251	49	H	H	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	436
252	-4	t-Bu	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	1	803
253	-5	t-Bu	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	1	811
254	-9	t-Bu	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_{10}NH$	1	787
255	0	t-Bu	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_{12}NH$	1	801
256	10	t-Bu	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	1	789

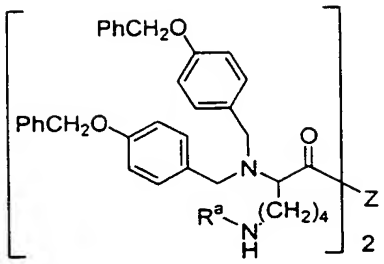


Table 13

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	Z	MS, $[MH_2/2]^+$
257	-26	Boc	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	731
258	-24	Boc	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	723
259	-13	Boc	$NH(CH_2)_{12}NH$	721
260	-12	Boc	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	695
261	51	H	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	595
262	93	$HO_2CCH_2CH_2CO$	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	695
263	88	$HO_2C(CH_2)_3CO$	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	709
264	89	$HO_2CCH_2CMe_2CH_2CO$	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	737
265	65	$HO_2CCH_2CH_2CO$	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	723
266	82	$HO_2C(CH_2)_3CO$	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	737
267	83	$HO_2CCH_2CMe_2CH_2CO$	$NH(CH_2)_3O(CH_2)_4O(CH_2)_3NH$	765
268	40	$HO_2CCH_2CMe2CH_2CO$	$NH(CH_2)_{12}NH$	764
269	55	$HO_2CCH_2CH_2CH_2CO$	$NH(CH_2)_{12}NH$	735
270	56	$HO_2CCH_2CH_2CO$	$NH(CH_2)_{12}NH$	721
271	77	$HO_2CCH_2CH_2CO$	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	731
272	78	$HO_2CCH_2CH_2CH_2CO$	$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NH$	745

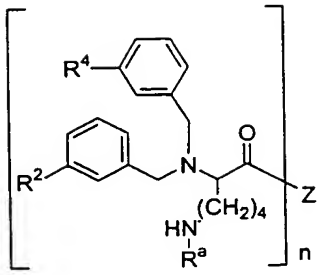


Table 14

cpd	EPO / EBP-Ig %inh @ 50 μM	R^a	R^2	R^4	Z	n	MS, $[MH_2/2]$ +
273	nd	$HO_2CCH_2CH_2CO$	4-Me-PhO	4-Me-PhO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	695
274	nd	$HO_2CCH_2CH_2CO$	PhO	PhO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	667
275	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhO	4-MeO-PhO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	727
276	nd	$HO_2CCH_2CH_2CO$	4-t-Bu-PhO	4-t-Bu-PhO	$NH(CH_2)_2O(CH_2)_2O(CH_2)_2NH$	2	780
277	nd	H	PhO	PhO	$(NHCH_2CH_2)_3N$	3	813
278	nd	H	4-Me-PhO	4-Me-PhO	$(NHCH_2CH_2)_3N$	3	855
279	nd	H	4-MeO-PhO	4-MeO-PhO	$(NHCH_2CH_2)_3N$	3	903
280	nd	$HO_2CCH_2CH_2CO$	4-MeO-PhO	4-MeO-PhO	$(NHCH_2CH_2)_3N$	3	1053
281	nd	$HO_2CCH_2CH_2CO$	4-Me-PhO	4-Me-PhO	$(NHCH_2CH_2)_3N$	3	1005
282	nd	$HO_2CCH_2CH_2CO$	PhO	PhO	$(NHCH_2CH_2)_3N$	3	963
283	nd	Boc	PhO	PhO	$NH(CH_2)_3NMe(CH_2)_3NH$	2	666
284	nd	Boc	4-Me-PhO	4-Me-PhO	$NH(CH_2)_3NMe(CH_2)_3NH$	2	694

5

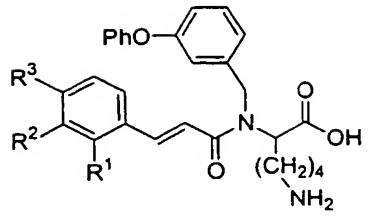


Table 15

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	MS, MH+
285	-28	Me	H	H	473
286	46	H	BnO	H	565
287	36	H	4-Me-PhO	H	565
288	27	H	4-tBu-PhO	H	607
289	20	H	H	PhO	551

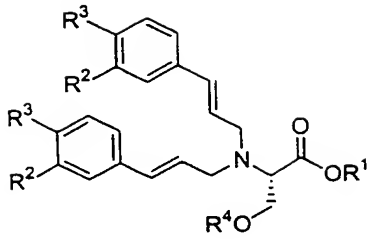


Table 16A

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	R^4
290	0	Me	PhO	H	t-Bu
291	17	Me	H	Ph	t-Bu
292	11	Me	3-CF3-C6H4O	H	t-Bu
293	14	Me	3,4-Cl2-C6H3O	H	t-Bu
294	9	Me	4-t-Bu-C6H4O	H	t-Bu
295	10	Me	H	Ph	H
296	0	Me	3,4-Cl2-C6H3O	H	H
297	0	Me	3-CF3-C6H4O	H	H
298	1	Me	4-t-Bu-C6H4O	H	H
299	nd	Me	PhO	H	H
300	57	H	PhO	H	H
301	25	H	H	Ph	t-Bu
302	30	H	3,4-Cl2-C6H3O	H	t-Bu
303	21	H	3-CF3-C6H4O	H	t-Bu
304	19	H	4-t-Bu-C6H4O	H	t-Bu
305	48	H	H	Ph	H
306	21	Me	H	H	t-Bu

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	R^4
307	25	H	3,4-Cl2-C6H3O	H	H
308	25	H	3-CF3-C6H4O	H	H
309	13	H	4-t-Bu-C6H4O	H	H
310	34	Me	H	H	H

Table 16B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
290	10-30% EtOAc/hex	pale yellow oil	C38H41NO5	592
291	1:5 EtOAc/hex	yellow oil	C38H41NO3	560
292	1:5 EtOAc/hex	yellow oil	C40H39F6NO5	728
293	1:5 EtOAc/hex	yellow oil	C38H37Cl4NO5	728
294	1:5 EtOAc/hex	yellow oil	C46H57NO5	704
295		off-white solid	C34H33NO3 / 1 C2H4O2	504
296		amber solid	C34H29Cl4NO5 / 1 C2H4O2	672
297		amber oil	C36H31F6NO5 / 1 C2H4O2	672
298		off-white solid	C42H49NO5 / 1 C2H4O2	648
299	30% EtOAc/hex	colorless oil	C34H33NO5	536
300		sticky yellow solid	C33H31NO5 / 1 C2H4O2	522
301		yellow solid	C37H39NO3	546
302		amber oil	C37H35Cl4NO5	714

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
303		amber oil	C39H37F6NO5	714
304		amber oil	C45H55NO5	690
305		amber solid	C33H31NO2 / 1 C2HF3O2	490
306		light-yellow oil	C26H33NO3 / 0.25 H2O	408
307		amber solid	C33H27Cl4NO5 / 1 C2HF3O2	658
308		amber oil	C35H29F6NO5 / 1 C2HF3O2	658
309		off-white solid	C41H47NO5 / 1 C2HF3O2	634
310		light yellow oil	C22H25NO3 / 1 C2H4O2	352

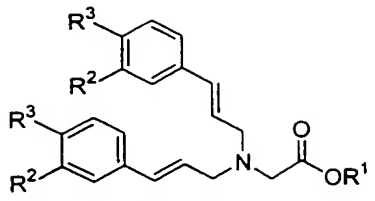


Table 17A

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3
311	5.3	t-Bu	PhO	H
312	45	H	PhO	H

Table 17B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
311	10% EtOAc/hex	pale yellow oil	C36H37NO4	548
312		sticky brown solid	C32H29NO4 / 1 C2HF3O2	492

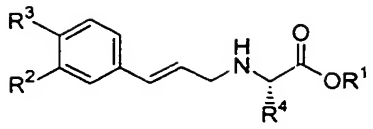


Table 18A

cpd	EPO / EBP-Ig, %inh @ 50 μM	R^1	R^2	R^3	R^4
313	28	H	H	CF3	(CH2)4NH(2-Cl-Cbz)
314	12	Me	H	CO2H	(CH2)4NH2
315	nd	Me	H	NO2	(CH2)4NHBoc
316	20	Me	OPh	H	(CH2)4NHBoc
317	13	Me	4-t-Bu-C6H4O	H	(CH2)4NHBoc
318	14	Me	H	H	(CH2)4NHCbz
319	nd	Me	H	H	(CH2)4NHCbz
320	17	Me	H	OMe	(CH2)4NHCbz
321	42	Me	CO2Me	H	(CH2)4NHCbz
322	nd	Me	H	2,3-benzo	(CH2)4NHCbz
323	6	Me	H	CO2H	(CH2)4NHCbz
324	nd	Me	H	CO2Me	nd

Table 18B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
313		yellow oil	C25H28ClF3N2O4 \ 1 C2HF3O2	499
314		yellow oil	C17H24N2O4	321
315	30% EtOAc/hex	dark yellow gum	C21H31N3O6	422
316	20-50% EtOAc/hex	pale yellow oil	C27H36N2O5	469
317		pale yellow oil	C31H44N2O5	525
318		gum	C24H30N2O4	411

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
319		pale yellow oil	C24H30N2O4	411
320	2% MeOH/CH2Cl2	yellow oil	C25H32N2O5	441
321		yellow oil	C26H32N2O6 \ 1 C2H4O2	469
322	25-50% EtOAc/hex	clear residue	C28H32N2O4	461
323		yellow oil	C25H30N2O6	455
324		yellow oil	C26H32N2O6	469

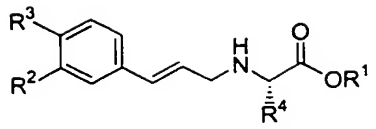


Table 19A

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	R^4
325	6	Me	OPh	H	CH2CH2CO2H
326	0	H	OPh	H	CH2CH2CO2H
327	11	Me	H	Ph	CH2CH2CO2H
328	33	Me	3,4-Cl2-C6H3O	H	CH2CH2CO2H
329	13	H	H	Ph	CH2CH2CO2H
330	12	H	3-CF3-C6H4O	H	CH2CH2CO2H
331	18	H	4-t-Bu-C6H4O	H	CH2CH2CO2H
332	17	H	3,4-Cl2-C6H3O	H	CH2CH2CO2H
333	16	Me	3,4-benzo		CH2CH2CO2-t-Bu
334	6	Me	OPh	H	CH2CH2CO2-t-Bu
335	25	Me	H	Ph	CH2CH2CO2-t-Bu
336	32	Me	3,4-Cl2-C6H3O	H	CH2CH2CO2-t-Bu
337	0	H	OPh	H	CH2CH2CO2-t-Bu
338	23	t-Bu	3-CF3-C6H4O	H	CH2CH2CO2-t-Bu
339	10	t-Bu	4-t-Bu-C6H4O	H	CH2CH2CO2-t-Bu
340	14	H	H	Ph	CH2CH2CO2-t-Bu
341	19	H	3,4-Cl2-C6H3O	H	CH2CH2CO2-t-Bu

Table 19B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
325		off-white solid	C21H23NO5 \ 1 C2F3HO2	370
326		fluffy white solid	C20H21NO5 \ 1 C2HF3O2	356
327		off-white solid	C21H23NO4 \ 1 C2F3HO2	354
328		amber oil	C21H21Cl2NO5 \ 1 C2F3HO2	438
329		amber solid	C20H21NO4 \ 1 C2HF3O2	340
330		amber oil	C21H20F3NO5 \ 1 C2HF3O2	424
331		amber oil	C24H29NO5 \ 1 C2HF3O2	412
332		amber oil	C20H19CL2NO5 \ 1 C2HF3O2	424
333	10-25% EtOAc/hex	yellow oil	C23H29NO4	384
334	10-30% EtOAc/hex	pale yellow oil	C25H31NO5	426
335	1:5 EtOAc/hex	yellow oil	C25H31NO4	410
336	1:5 EtOAc/hex	yellow oil	C25H29Cl2NO5	494
337		white powder	C24H29NO5 \ 0.4 H2O	412
338	1:5 EtOAc/hex	yellow oil	C29H36F3NO5	536
339	1:5 EtOAc/hex	yellow oil	C32H45NO5	524
340		yellow solid	C24H29NO4	396
341		white solid	C24H27Cl2NO5	480

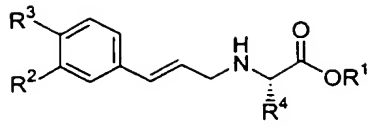


Table 20A

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	R^4
342	0	Me	H	Ph	CH2OH
343	37	Me	4-t-Bu-C6H4O	H	CH2OH
344	4	Me	3-CF3-C6H4O	H	CH2OH
345	40	Me	3,4-Cl2-C6H3O	H	CH2OH
346	28	Me	OPh	H	CH2OH
347	23	H	OPh	H	CH2OH
348	21	H	H	Ph	CH2OH
349	23	H	3,4-Cl2-C6H3O	H	CH2OH
350	23	H	3-CF3-C6H4O	H	CH2OH
351	29	H	4-t-Bu-C6H4O	H	CH2OH
352	8	Me	OPh	H	CH2O-t-Bu
353	24	Me	H	Ph	CH2O-t-Bu
354	31	Me	3,4-Cl2-C6H3O	H	CH2O-t-Bu
355	22	Me	3-CF3-C6H4O	H	CH2O-t-Bu
356	23	Me	4-t-Bu-C6H4O	H	CH2O-t-Bu
357	12	H	3-CF3-C6H4O	H	CH2O-t-Bu

Table 20B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
342		off-white solid	C19H21NO3 \ 1 C2F3HO2	312
343		amber oil	C23H29NO4 \ 1 C2F3HO2	384
344		amber oil	C20H20F3NO4 \ 1 C2F3HO2	396
345		amber oil	C19H19Cl2NO4 \ 1 C2F3HO2	396
346	EtOAc	pale yellow oil	C19H21NO4	328
347		amber oil	C18H19NO4 \ 1 C2HF3O2	314
348		yellow solid	C18H19NO3 \ 1 C2HF3O2	298
349		amber oil	C18H17Cl2NO4 \ 1 C2HF3O2	382
350		amber oil	C19H18F3NO4 \ 1 C2HF3O2	382
351		amber oil	C22H27NO4 \ 1 C2HF3O2	370
352	10-30% EtOAc/hex	pale yellow oil	C23H29NO4	384
353	20% EtOAc/hex	off-white solid	C23H29NO3	368
354	20% EtOAc/hex	yellow oil	C23H27Cl2NO4	452
355	20% EtOAc/hex	yellow oil	C24H28F3NO4	452
356	20% EtOAc/hex	yellow oil	C27H37NO4	440
357		white solid	C23H26F3NO4	438

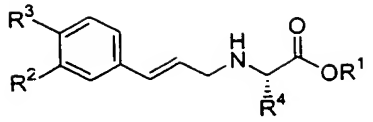


Table 21A

cpd	EPO / EBP-Ig %inh @ 50 μM	R^1	R^2	R^3	R^4
358	0	H	H	CF3	(s)-CH(OH)CH3
359	25	Me	CO2Me	H	(s)-CH(OMe)CH3
360	18	Me	H	H	Bn
361	24	Me	CO2Me	H	Bn
362	0	H	H	CF3	CH2(4-HOC6H4)
363	33	Me	CO2Me	H	CH2(4-MeOC6H4)
364	16	Me	H	H	CH2(indol-3-yl)
365	0	H	H	CF3	CH2CH2SMe
366	38	Me	CO2Me	H	CH2CO2Me
367	0	H	H	CF3	CH2CONH2
368	40	Me	CO2Me	H	CH2SBn
369	12	H	H	CF3	i-Bu
370	0	H	H	CF3	i-Pr
371	16	Me	CO2Me	H	i-Pr
372	0	Me	H	H	Me

Table 21B

cpd	MPLC solvent	appearance	empirical formula	MS, MH+
358		amber oil	C14H16F3NO3 \ 1 C2HF3O2	304
359		amber oil	C17H23NO5 \ 1 C2H4O2	322
360	20% EtOAc/hex	light-yellow oil	C19H21NO2	296
361		amber oil	C22H25NO5 \ 1 C2H4O2	354
362		amber oil	C19H18F3NO3 \ 1 C2HF3O2	366
363		amber oil	C22H25NO5 \ 1 C2H4O2	384
364	1:2 EtOAc/hex	tan solid	C21H22N2O2	335
365		amber oil	C15H18F3NO2S \ 1 C2HF3O2	334
366		amber oil	C17H21NO6 \ 1 C2H4O2	336
367		amber oil	C14H15F3N2O3 \ 1 C2HF3O2	317
368		amber oil	C22H25NO4S \ 1 C2H4O2	400
369		amber oil	C17H22F3NO2 \ 1 C2HF3O2	316
370		amber oil	C15H18F3NO2 \ 1 C2HF3O2	302
371		amber oil	C17H23NO4 \ 1 C2H4O2	306
372	20% EtOAc/hex	yellow oil	C13H17NO2 \ 0.10 C4H8O2	220